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DEGREE OF RESEMBLANCE OF PARENTS AND OFFSPRING WITH RESPECT TO BIRTH AS TWINS FOR REGISTERED SHROPSHIRE SHEEP

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INTRODUCTION

In 1906 Rommel and Phillips[1] reported an investigation into the inheritance of the size of litter in the female line in Poland China sows based on data of the American Poland China Record. In 1907 there was published a statistical analysis of data from the records of Weldon[2] on heredity in the size of litters in mice.

The present investigation resembles somewhat the above investigations in that it is concerned with the question of the likeness of animals and their offspring with respect to being born as singles, twins, or triplets. We are concerned with the question whether and to what extent the offspring of parents born in twins and of grandparents born in twins are more likely to be twins than if these ancestors are born as singles.

Stated in another way, when we know that certain animals of a given breed or class and certain of their ancestors are born as twins or triplets, is there greater probability that the offspring will be twins or triplets than if the animals were born as singles?

Our problem may be made clearer by asking a question that covers only part of the problem: Does the subclass of sheep of a certain breed which consists of those born as twins tend to beget twins in larger proportion than the subclass that consists of those born as singles?

In the Journal of the Royal Agricultural Society of England[3] there appears a statement of some of the possible causes that are favorable to the production of twins. It is there stated that "there is some reason to believe that twin lambs produce more twins than single lambs, and that the influence of heredity is brought to bear." The main purpose

[1] Rommel, G. M., and Phillips, E. F. Inheritance in the female line of size of litter in Poland China sows. *In* Biometrika, v. 5, pt. 1/2, p. 203–205. 1906.

[2] [Weldon, W. F. R.] On heredity in mice from the records of the late W. F. R. Weldon. *In* Biometrika, v. 5, pt. 4, p. 436–449. 1907.

[3] Heape, Walter. Abortion, barrenness, and fertility in sheep; an abstract of records obtained for the year 1896–97. *In* Jour. Roy. Agr. Soc. England, s. 3, v. 10, pt. 2, p. 236. 1899.

of the present paper is to submit an analysis of data with a view to testing the foundations of such a belief when we are dealing with a class of pure-bred sheep.

To indicate birth as a single, in twins, or in triplets, we use the symbols "1" for single, "2" for twin, and "3" for triplet.

SOURCE OF DATA

The source of all our data is the American Shropshire Sheep Record. We have taken individuals with numbers from 325502 to 344869,[1] and have looked up their parents and grandparents with respect to the state of birth in singles, twins, and triplets.

All cases are omitted where either parent is imported, for the reason that the English records do not show whether an animal is born single, in twins, or in triplets.

For each of the offspring above mentioned, with American-born parents, we have made a card, showing whether this animal is born in our symbolism as a 1, 2, or 3 and showing in which of the states 1, 2, or 3 its parents and nonimported grandparents are born.

DISCRIMINATION IN FAVOR OF OR AGAINST RECORDING TWINS

In beginning this investigation we made some inquiry concerning possible discrimination in favor of or against the recording of twins, and found no reason to believe that there existed such discrimination. In the Journal of the Royal Agricultural Society of England,[2] we find that 46.84 per cent of Shropshire ewes involved in the data there analyzed have twins. This would mean that nearly 64 per cent of the lambs born are twins. But the percentage of lambs born as twins and triplets that we have found in fairly large classes of offspring does not seem to exceed 43, which is very different from 64 per cent. The difference seems to mean that either Shropshires in America are less fertile than in England or there is discrimination against twins in the matter of recording. This does not mean that the discrimination is made directly against twins, but probably in an indirect manner for some such reason as the better development of singles when the selections are made. However, for the main purpose of our problem, we are concerned with the elimination of discriminations where one would record twins or singles because certain ancestors are twins or singles—that is, we are concerned with the elimination of the kind of discrimination that would give an affirmative answer to the question, Do breeders tend to record an increased or decreased proportion of twins on account of the fact that certain parts of the ancestry consist of twins or of singles? Such discrimination is doubtless much less likely than a more general sort of discrimination that would lead to the recording of a larger or smaller proportion of twins than we find in a random sample.

[1] American Shropshire Sheep Record, v. 25, p. 1–1314. 1912. [2] Heape, Walter. Op. cit., p. 235.

There are some cases where owners of sires of large production have recorded from the same sire in two consecutive years all singles in one of the years and nearly all twins in the other year. Such cases make it appear that these few owners tend to select twins or singles in making records. The following section, "Repetition of sires and paternal grandparents," shows how we have treated such special cases.

REPETITION OF SIRES AND PATERNAL GRANDPARENTS

One sire may belong to a large number of recorded offspring, although this happens in relatively few cases; for example, there is a case in which one sire belongs to as many as 135 recorded offspring in the period for which we have examined offspring, and this fact is not to be neglected in making a critical examination of our data. In fact, a few such cases of large production with discrimination against either twins or singles might vitiate our results on the correlation of offspring with sires and grandsires. On account of the possibility of error from this source, we arrange a table, separating the offspring of each sire into singles and twins. From this arrangement of data it is fairly clear that certain cases of extreme percentages of singles or twins from a sire of large production should be excluded from data used in the calculations of statistical constants. We fix criteria somewhat arbitrarily as follows:

(1) Cases are excluded where a sire has more than 10 recorded offspring that are all singles or all twins.

(2) Cases are excluded where a sire has more than 20 recorded offspring if the *difference* in percentage of twins among this offspring and among the general population of offspring is more than three times the probable error of the difference.

While we exclude from our calculations the part of the data just mentioned, we give such data in Tables XV, XVI, XVII, XXXV, and XXXVI, in order that anyone who may consider the above criteria for exclusion too stringent or too lenient may have available for criticism such excluded data. Tables I, IX to XIV, XVIII, and XXVI to XXXII involve data about sires and do not include data concerning those cases of high-producing sires which are to be excluded from our calculations of statistical constants.

ANALYSIS OF DATA FOR SIRES, DAMS, AND OFFSPRING

Table I(A) shows the frequencies with which sires and dams born in states 1–1, 1–2, 2–1, 2–2, 1–3, 3–1, 2–3, 3–2, or 3–3 beget recorded offspring born in states 1, 2, or 3. In this notation the first number of a pair refers to the sire and the second to the dam. Thus, 1–2 means that the sire is born as a single and the dam as a twin. To illustrate further the meaning of the table, consider the number 1,276 in the column marked "1" and in the row marked "2–1." This means that 1,276 offspring out of the total of 9,291 are singles with twin sires and single dams.

TABLE I(A).—*Correlation between size of litter in which offspring are born and size of litter in which pairs of parents are born*

Sires and dams.	Offspring.			Total.
	1	2	3	
1–1	2,018	1,026	15	3,059
1–2	1,430	996	10	2,436
2–1	1,276	800	12	2,088
2–2	867	661	22	1,550
1–3	24	29	.....	53
3–1	3	14	.....	17
2–3	21	20	3	44
3–2	10	21	2	33
3–3	9	2	.....	11
Total	5,658	3,569	64	9,291

$$r = 0.0880 \pm 0.0070,$$

where r is the correlation coefficient between the sum of numbers in litters in which sire and dam are born and the number in corresponding litters in which offspring are born.

Means of arrays of offspring:

(1) When sire and dam are singles..... 1.3452±0.0059.
(2) When sire is single and dam is twin..... 1.4171±0.0067.
(3) When sire is twin and dam is single..... 1.3946±0.0073.
(4) When sire is twin and dam is twin..... 1.4548±0.0088.
(5) When either sire or dam is a triplet..... 1.6076±0.030.
Mean of all offspring..... 1.3979±0.0035.

Table I(B) simply exhibits percentages of singles, twins, and triplets among offspring that belong to different kinds of parents. It seems from this table that the percentage of twins among offspring of twin parents is greater than that among the offspring of single parents. From the means of arrays of offspring in Table I(A), we note that the differences are significant when judged by their probable errors. The correlation between the sum of the numbers of lambs born in the litters in which the two parents are born and the number in the litter in which the offspring is born, given by

$$r = 0.0880 \pm 0.0070,$$

is a significant positive correlation.

TABLE I(B).—*Percentages of offspring born in states 1, 2, and 3, to correspond to states 1–1, 1–2, 2–1, . . . of sire and dam*

Sires and dams.	Offspring.		
	1	2	3
1–1	65.97	33.54	0.50
1–2	58.70	40.89	.41
2–1	61.11	38.31	.58
2–2	55.93	42.65	1.42
(a)	42.40	54.43	3.17
Based on total	60.90	38.41	.69

a This row of percentages is obtained by grouping together all offspring where either parent is a triplet. Even when thus grouped, the number of offspring is small.

TABLES FOR DAMS AND OFFSPRING

Table II(A) shows the frequencies with which recorded offspring born in states 1, 2, and 3 have dams born in these states, or the frequencies with which dams born in states 1, 2, or 3 beget recorded offspring in states 1, 2, or 3.

TABLE II(A).—*Correlation between size of litter in which offspring are born and size of litter in which dams are born*

Dams.	Offspring.			Total.
	1	2	3	
1	3,784	2,046	27	5,857
2	2,586	1,930	40	4,556
3	58	58	3	119
Total	6,428	4,034	70	10,532

$r = 0.0869 \pm 0.0065$.

Means of arrays of offspring:

(1) When dams are singles. . . . . 1.3585 ± 0.0043.
(2) When dams are twins. . . . . 1.4412 ± 0.0051.
(3) When dams are triplets. . . . . 1.538 ± 0.034.

Table II(B) simply converts into the form of percentages the frequencies given in Table II(A), so that the essential points of interest may be grasped more easily. From means of arrays of offspring in Table II(A), we note that there is a significant tendency for twin dams to produce a larger percentage of twins than is produced by single dams. The correlation is given by

$$r = 0.0869 \pm 0.0065.$$

TABLE II(B).—*Percentages of offspring born in states 1, 2, and 3 to correspond to states 1, 2, and 3 of the dams*

Dams.	Offspring.		
	1	2	3
1	64.61	34.93	0.46
2	56.76	42.36	.88
3	48.7	48.7	2.5

ANALYSIS OF DATA FOR DAMS, MATERNAL GRANDPARENTS, AND OFFSPRING

Table III(A) represents the distribution of offspring, dams, and maternal granddams with respect to states 1, 2, and 3. To illustrate the meaning of the table, consider the number 1,393 in the column headed 1 and in the row marked 1–2. This means that there are 1,393 of the

total number of offspring that are singles with single dams and twin maternal granddams.

Dams and granddams are repeated so as to let all the recorded offspring appear. Since we do not know in which of the states 1, 2, or 3 imported sheep were born, we have to omit all cases of imported granddams.

TABLE III(A).—*Correlation between offspring and maternal granddams*[a]

Dams and maternal granddams.	Offspring.			Total.
	1	2	3	
1–1	2,170	1,098	17	3,285
1–2	1,393	796	7	2,196
2–1	1,312	935	12	2,259
2–2	1,126	881	22	2,029
1–3	34	30	.....	64
3–1	19	20	1	40
2–3	27	29	4	60
3–2	26	21	2	49
3–3	3	8	.....	11
Total	6,110	3,818	65	9,993

[a] This abbreviated heading will be used for tables that follow. The correlation refers to that between sizes of litters in which the classes are born.

Means of arrays of offspring:

(1) When dams and granddams are singles..... 1.3446±0.0057.
(2) When the dams are singles and granddams twins.... 1.3689±0.0070.
(3) When the dams are twins and granddams are singles. 1.4245±0.0071.
(4) When the dams are twins and granddams are twins.. 1.4559±0.0078.
(5) When either dam or granddam is a triplet..... 1.545 ±0.037.

Table III(B) exhibits the percentages of offspring born in states 1, 2, and 3 for dam and maternal granddams born in various states.

TABLE III(B).—*Percentages of offspring in states 1, 2, and 3 to correspond to states 1–1, 1–2, 2–1, 2–2, of dams and maternal granddams*

Dams and maternal granddams.	Offspring.		
	1	2	3
1–1	66.06	33.42	0.52
1–2	63.43	36.25	.32
2–1	58.08	41.39	.53
2–2	55.50	43.42	1.08
(a)	48.66	48.21	3.13

[a] See footnote, Table I(H).

It is to be noted from Table III(B) that the percentage of twins varies from 33.42 to 48.21, there being a gradual increase in percentage of twins when twins and triplets occur as dams and granddams. The greatest influence we should ascribe to the twin dam, as the occurrence of the twin granddam does not increase the percentage nearly as much as does that of the twin dam.

We hesitate to calculate a correlation coefficient from Table III(A), for the reason that we have no single number to mark each selected array and would not feel justified in giving an interpretation to a correlation coefficient obtained by taking the sum or mean value of the two numbers associated with each array. We might weight the dam and granddam, but such a system of weights would in the present state of knowledge be little better than a set of guesses.

We may note from the means of arrays and their probable errors that there can be no reasonable doubt about the significance of the difference in means when in one case the dams are singles and in the other they are twins. Further, it is to be observed that there appears to be a slight influence of twin maternal granddams in increasing mean values. Such influence is not, however, nearly so surely established as is the influence of the dams. In fact, the difference in the case of single and twin granddams with single dams is not quite three times the probable error.

Table IV shows the frequencies with which maternal granddams born in states 1, 2, or 3 beget recorded offspring born in states, 1, 2, or 3. There is a significant correlation given by

$$r = 0.0433 \pm 0.0067.$$

TABLE IV.—*Correlation between offspring and maternal granddams*

Maternal granddams.	Offspring.			Total.
	1	2	3	
1	3,501	2,033	30	5,584
2	2,545	1,698	31	4,274
3	64	67	4	135
Total	6,110	3,818	65	9,993

Means of arrays of offspring:

(1) When the maternal granddams are singles..... 1.3784±0.0045.
(2) When the maternal granddams are twins..... 1.4120±0.0052.
(3) When the maternal granddams are triplets..... 1.556 ±0.033.

Table V(A) represents the distribution of offspring, dams, and maternal grandsires with respect to states 1, 2, and 3 in a manner analogous to that of Table III(A). Here again, when dams are twins, we note

from the percentages in Table V(B) that the percentage of twins increases, but there is no significant difference between the percentages of twins produced by single dams whose sires are singles and by single dams whose sires are twins. Further, there appears to be no significant difference between the percentages of twins produced by twin dams whose sires are singles and by twin dams whose sires are twins. Further, Table VI shows the frequencies with which maternal grandsires born in states 1, 2, or 3 beget offspring born in states 1, 2, or 3. From this table we obtain the correlation coefficient

$$r = 0.0042 \pm 0.0073,$$

which is so small that we are unable to assert the existence of any significant correlation.

TABLE V(A).—*Correlation between offspring and maternal grandsires*

Dams and maternal grandsires.	Offspring.			Total.
	1	2	3	
1–1	1,751	929	2	2,682
1–2	1,159	596	13	1,768
2–1	1,159	894	17	2,070
2–2	818	596	19	1,433
1–3	38	27	1	66
3–1	20	32	3	55
2–3	25	21	1	47
3–2	21	18	.....	39
3–3	.....	.....	.....	.....
Total	4,991	3,113	56	8,760

Table V(A) does not include so many records as previous tables because imported grandsires can not be included.

TABLE V(B).—*Percentages of offspring in states 1, 2, and 3 to correspond to states 1–1, 1–2, 2–1, ... of the dams and maternal grandsires*

Dams and maternal grandsires.	Offspring.		
	1	2	3
1–1	65.29	34.64	0.07
1–2	65.55	33.71	.74
2–1	55.99	43.19	.82
2–2	57.08	41.59	1.33
(*a*)	50.24	47.34	2.42
Based on total	61.16	38.15	.69

[a] See footnote, Table I (B).

TABLE VI.—*Correlation between offspring and maternal grandsires*

Maternal grandsires.	Offspring.			Total.
	1	2	3	
1	2,930	1,855	22	4,807
2	1,998	1,210	32	3,240
3	63	48	2	113
Total	4,991	3,113	56	8,160

$r=0.0042 \pm 0.0073$.

Means of arrays of offspring:

(1) When maternal grandsires are singles ..... 1.3950±0.0059.
(2) When maternal grandsires are twins..... 1.3932±0.0049.
(3) When maternal grandsires are triplets..... 1.460 ±0.034.

Tables VII(A) and VII(B) are correlation tables for maternal granddams and dams. These tables show correlation between dams and offspring just as Tables II(A) and II(B) do, the only difference being that the offspring is in this case limited to the females who themselves become dams.

TABLE VII(A).—*Correlation between dams and maternal granddams*

Maternal granddams.	Dams.			Total.
	1	2	3	
1	2,754	1,813	37	4,604
2	1,810	1,570	45	3,425
3	54	45	9	108
Total	4,618	3,428	91	8,137

$r=0.0770 \pm 0.0074$.

TABLE VII(B).—*Percentages of dams in states 1, 2, and 3 to correspond to states 1, 2, and 3 of the maternal granddams*

Maternal granddams.	Dams.		
	1	2	3
1	59.82	39.38	0.80
2	52.85	45.84	1.31
3	50.00	41.67	8.33
Based on total	56.75	42.13	1.12

The correlation coefficient computed from the data of Table VII (A) is

$$r = 0.0770 \pm 0.0074.$$

This correlation is significant when judged by its probable error, and it does not differ significantly from the correlation between dams and offspring as found from Table II(A).

Table VIII(A) is a correlation table for maternal grandsires and dams. This is the case of the correlation of sires with female offspring with respect to birth as singles, twins, or triplets. The correlation coefficient is

$$r = 0.0066 \pm 0.0075.$$

This result makes it somewhat probable that there is no correlation between sires and their female offspring with respect to the character in question. We shall investigate this matter further by the separation of offspring with regard to sex. (See Tables XXV and XXXI.)

TABLE VIII(A).—*Correlation between dams and maternal grandsires*

Maternal grandsires.	Dams.			Total.
	1	2	3	
1	2,255	1,665	45	3,965
2	1,514	1,145	31	2,690
3	57	48	.....	105
Total	3,826	2,858	76	6,760

$r = 0.0066 \pm 0.0075$.

TABLE VIII(B).—*Percentages of dams in states 1, 2, and 3 to correspond to states 1, 2, and 3 of the maternal grandsires*

Maternal grandsires.	Dams.		
	1	2	3
1	56.87	41.99	1.14
2	56.28	42.57	1.15
3	54.29	45.71	.....
Based on total	56.60	42.28	1.12

ANALYSIS OF DATA FOR SIRES AND OFFSPRING

Tables IX(A) and IX(B) are analogous to Tables II(A) and II(B), where the dams are replaced by sires and where certain abnormal cases are excluded in accord with criteria given under "Repetition of sires and paternal grandparents."

TABLE IX(A).—*Correlation between offspring and sires*

Sires.	Offspring.			Total.
	1	2	3	
1	3,472	2,051	25	5,548
2	2,164	1,479	37	3,680
3	22	39	2	63
Total	5,658	3,569	64	9,291

$r = 0.0527 \pm 0.0070.$

Means of arrays of offspring:

(1) When the sire is a single ..... 1.5787 ± 0.0045.
(2) When the sire is a twin ..... 1.4220 ± 0.0057.
(3) When the sire is a triplet ..... 1.6825 ± 0.045.

TABLE IX(B).—*Percentages of offspring in states 1, 2, and 3 to correspond to states 1, 2, and 3 of the sires*

Sires.	Offspring.		
	1	2	3
1	62.58	36.97	0.45
2	58.80	40.19	1.01
3	34.92	61.90	3.17
Based on total	60.90	38.41	0.69

While the same sort of tendencies are to be noted in the examination of these tables as with Tables II(A) and II(B), it seems that the tendency of twins to produce twins is less marked and that the correlation is given by

$$r = 0.0527 \pm 0.0070.$$

If we had excluded none of the offspring of large producing sires with an abnormal proportion of recorded twins or singles, we should have obtained

$$r = 0.0294 \pm 0.0066.$$

We think there is, however, little doubt that it would be improper to include for purposes of calculation at least most of the cases we have excluded.

We may well note the increase in means of arrays for twin and triplet sires.

In order to examine a little more critically into the question whether twin sires tend to have a larger number of twin offspring than do single sires, we have invented an index number for each sire. This number is

formed by marking with a "1" each of the offspring born single and with a "2" each one born in twins and next by finding the arithmetical mean of the numbers that belong to the offspring of any sire. Clearly, if all the offspring of a sire were singles, under this scheme his index number would be 1, while if all were born in twins, his index number would be 2.

Next, finding the arithmetical mean of the index numbers for all the sires, we have a sort of measure that enables us to compare the tendency of twins to produce twins and without giving weight to repetitions of the sires to correspond to each individual offspring.

It results that the following numbers are associated with sires born as singles and sires born twins.

For all sires born in singles, we have the mean value 1.3300 ± 0.0078.

For all sires born in twins, we have the mean value 1.380 ± 0.010.

These results show a larger relative production of twins by twin sires than by single sires. The difference, however, is only about four times the probable error of the difference. This surely means that it takes rather large numbers to establish the significance of the difference if such difference exists.

In making the application of this scheme of index numbers, we came upon an interesting form of frequency distribution that seems to occur but rarely. In these distributions the most frequent values are at the index numbers 1 and 2. The modal values are thus at or near the ends of the range. The following are the distributions:

Sires born in singles.		Sires born in twins.	
Index number.	Frequency.	Index number.	Frequency.
1.0	586	1.0	342
1.1	19	1.1	14
1.2	57	1.2	19
1.3	66	1.3	56
1.4	51	1.4	33
1.5	72	1.5	49
1.6	47	1.6	25
1.7	68	1.7	52
1.8	30	1.8	26
1.9	27	1.9	11
2.0	170	2.0	126
2.3	1	2.1	2
2.5	2	2.3	1
3.0	4	2.4	1
		2.5	2
		3.0	8

The large numbers at or near the ends of the range are to be accounted for, in part at least, by the fact that a considerable number of sires have only one recorded offspring. In such cases the index number must be 1 or 2. It can not have an intermediate value. Next, with only a few

offspring recorded, say, with two individual offspring recorded, the index number may fall only into one of the three classes, 1, 2, or 1.5, when we neglect the rare case of triplets. For index numbers approaching 1 but not equaling 1, a larger number of offspring is required; for example, it would require at least 7 offspring, 6 born in singles and 1 in twins, to enter the class 1.1, and it would require at least 6 twins and 1 single to enter class 1.9.

These facts seem to account for the smaller numbers contiguous to the ends of the range than are found at other intermediate points.

ANALYSIS OF DATA ON SIRES AND PATERNAL GRANDPARENTS

Tables X (A) and X (B) represent the distribution of paternal granddams and sires of offspring treated in Tables II and IX. The means of arrays show that the tendency to produce twins is increased by the use of twin sires instead of singles sires, but that this tendency is not changed significantly by the use of twin paternal granddams instead of single paternal granddams.

This result is further supported by obtaining from Table XI(A) for the correlation coefficient

$$r = -0.0100 \pm 0.0084.$$

We are thus unable to assert the existence of a significant correlation.

TABLE X(A).—*Correlation between offspring and paternal granddams*

Sires and paternal granddams.	Offspring.			Total.
	1	2	3	
1-1	1,574	937	15	2,526
1-2	1,209	742	8	2,019
2-1	855	593	26	1,474
2-2	894	578	7	1,479
1-3	43	26	1	70
3-1	20	21	2	43
2-3	36	32	.....	68
3-2	2	21	.....	23
3-3	.....	.....	.....	.....
Total	4,693	2,950	59	7,702

Means of arrays of offspring:

(1) When sire and paternal granddam are singles..... 1.3828±0.0067.
(2) When sire is a single and paternal granddam is a twin. 1.3754±0.0072.
(3) When sire is a twin and paternal granddam is a single. 1.4376±0.0093.
(4) When sire is a twin and paternal granddam is a twin. 1.4003±0.0082.
(5) When either sire or paternal granddam is a triplet... 1.520 ±0.025.

TABLE X(B).—*Percentages of offspring in states 1, 2, and 3 to correspond to states 1–1, 1–2, 2–1, . . . of the sires and paternal granddams*

Sires and paternal granddams.	Offspring.		
	1	2	3
1–1	62. 31	37. 09	0. 60
1–2	62. 85	36. 75	. 40
2–1	58. 00	40. 23	1. 77
2–2	60. 45	39. 08	. 47
(a)	49. 51	49. 02	1. 47
Based on total	60. 93	38. 30	. 77

[a] See footnote, Table I(B).

TABLE XI(A).—*Correlation between offspring and paternal granddams*

Paternal granddams.	Offspring.			Total.
	1	2	3	
1	2, 449	1, 551	43	4, 043
2	2, 165	1, 341	15	3, 521
3	79	58	1	138
Total	4, 693	2, 950	59	7, 702

$r = -0.0100 \pm 0.0084$.

TABLE XI(B).—*Percentages of offspring in states 1, 2, and 3 to correspond to states 1, 2, and 3 of paternal granddams*

Paternal granddams.	Offspring.		
	1	2	3
1	60. 57	38. 36	1. 07
2	61. 49	38. 08	. 43
3	57. 2	42. 0	. 8

Tables XII(A) and XII(B) represent the distribution of paternal grandsires and sires of the offspring treated in Tables II and IX. Here again we note that there is no significant difference whether paternal grandsires are singles or twins. This conclusion is further supported by obtaining from Table XIII(A) the correlation coefficient

$$r = -0.0147 \pm 0.0097.$$

We are thus unable to assert the existence of a significant correlation.

TABLE XII(A).—*Correlation between offspring and paternal grandsires*

Sires and paternal grandsires.	Offspring.			Total.
	1	2	3	
1-1	1, 160	632	11	1, 803
1-2	732	362	10	1, 104
2-1	594	423	6	993
2-2	495	278	13	756
1-3	8	2		10
3-1	9	7	2	18
2-3	18	9	3	30
3-2				
3-3		4		4
Total	2, 956	1, 717	45	4, 718

Means of arrays:

(1) When sire and paternal grandsire are singles ..... 1. 3627 ± 0. 0077.
(2) When sire is single and paternal grandsire is a twin .. 1. 3460 ± 0. 0099.
(3) When sire is a twin and paternal grandsire is a single. 1. 4381 ± 0. 011.
(4) When sire is a twin and paternal grandsire is a twin. 1. 4021 ± 0. 013.
(5) When either sire or paternal grandsire is a triplet ... 1. 516 ± 0. 054.

TABLE XII(B).—*Percentages of offspring in states 1, 2, and 3 to correspond to states 1-1, 1-2, 2-1, . . . of the sires and grandsires*

Sires and paternal grandsires.	Offspring.		
	1	2	3
1-1	64. 34	35. 05	0. 61
1-2	66. 30	32. 79	. 91
2-1	56. 80	42. 60	. 60
2-2	61. 51	36. 77	1. 72
(a)	56. 45	35. 49	8. 66
Based on total	62. 65	36. 39	. 96

[a] See note, Table IIB).

TABLE XIII(A).—*Correlation between offspring and paternal grandsires*

Paternal grandsires.	Offspring.			Total.
	1	2	3	
1	1, 733	1, 062	19	2, 814
2	1, 197	640	23	1, 860
3	26	15	3	44
Total	2, 956	1, 717	45	4, 718

$r = -0.0147 \pm 0.0097$.

TABLE XIII(B).—*Percentages of offspring in states 1, 2, and 3 to correspond to states 1, 2, and 3 of the paternal grandsires*

Paternal grandsires.	Offspring.		
	1	2	3
1	61. 58	37. 74	0. 68
2	64. 35	34. 41	1. 24
3	59. 09	34. 09	6. 82
Based on total	62. 65	36. 39	.96

Tables XIV(A) and XIV(B) represent the result of pooling the data on parents and offspring included in Tables II(A), VII(A), VIII(A), and IX(A).

TABLE XIV(A).—*Correlation between offspring and parents, obtained by combining into one table the data of Tables II(A), VII(A), VIII(A), and IX(A)*

Parents.	Offspring.			Total.
	1	2	3	
1	12, 265	7, 575	134	19, 974
2	8, 074	6, 124	153	14, 351
3	191	190	14	395
Total	20, 530	13, 889	301	34, 720

$r=0.0597 \pm 0.0036$.

Means of arrays:

For parents..... 1. 4361 ± 0. 0020.

For offspring..... 1. 4174 ± 0. 0020.

TABLE XIV(B).—*Percentages of offspring in states 1, 2, and 3 to correspond to states 1, 2, and 3 of the parents*

Parents.	Offspring.		
	1	2	3
1	61. 40	37. 92	0. 68
2	56. 27	42. 67	1. 06
3	48. 35	48. 10	3. 55
Based on total	59. 13	40. 00	.87

It may be noted that the percentage of recorded twins produced by twin parents is larger than the percentage of recorded twins produced by single parents. The correlation coefficient between sizes of litters in which parents and offspring are born is given by

$$r=0.0597 \pm 0.0036.$$

It may also be noted that, on the whole, there is a slightly and significantly larger percentage of twins among parents than among recorded offspring. It must be remembered in this connection that the record of any parent is repeated a number of times equal to the number of the recorded offspring of the parent. Otherwise, one might be led to argue in a very obvious manner that if twinning is inherited and if there is no selection against twins, the percentage of twins in the offspring would exceed the percentage among parents, but such an argument is not valid when we repeat the parent, as above explained.

DATA NOT INCLUDED IN FOREGOING TABLES AND CALCULATIONS

Tables XV, XVI, and XVII exhibit data excluded from calculations of statistical constants because of the abnormal proportion of twins or singles recorded among the offspring of a single sire. The reasons and criteria for such exclusion are given under "Repetition of sires and paternal grandparents."

TABLE XV.—*Size of litter in which offspring are born and size of litter in which pairs of parents are born (from data excluded in making Tables I and IX)*

Sires and dams.	Offspring.			Total.
	1	2	3	
1–1	245	145		390
1–2	150	160	3	313
2–1	238	63		301
2–2	133	88	3	224
1–3	3	7		10
3–1	1	2		3
2–3				
3–2				
3–3				
Total	770	465	6	1,241

TABLE XVI.—*Sizes of litters in which offspring and sires and paternal granddams are born (from data excluded in making Tables X and XI)*

Sires and paternal granddams.	Offspring.			Total.
	1	2	3	
1–1	197	168		365
1–2	64			64
2–1	113	83	2	198
2–2	211	58		269
Total	585	309	2	896

TABLE XVII.—*Sizes of litters in which offspring and sires and paternal grandsires are born (from data excluded in making Tables XII and XIII)*

Sires and paternal grandsires.	Offspring.			Total.
	1	3	3	
1–1	51	28	.....	79
1–2	70	58	.....	128
2–1	111	35	.....	146
2–2	89	99	.....	188
1–3	15	.....	.....	15
Total	336	220	.....	556

DATA ON ANIMALS WITH A "COMPLETE AMERICAN" PEDIGREE

What we shall mean here by a "complete American" pedigree is a case where the offspring have parents and grandparents American-born, so that we have records as to whether these ancestors were born in states 1, 2, or 3. Statements are frequently made about the effects of climate on fertility; therefore we have thought that it would be of some interest to select from the total group of offspring that subgroup whose grandparents as well as parents are American-born. This requires that three generations at least be American born.[1] Further, the total subgroup of breeders who import a great deal may form a class that differs significantly in the handling of sheep from the totality of breeders who do not import.

This restriction to cases of "complete American" pedigree has brought our total number of cases down to only a little over one-third of the number treated in Tables I to XIV, but it seems worth while to present the tables showing corresponding frequencies of those with "complete American" pedigrees and to make some comparisons.

Any pedigree that has any grandparent not American-born is called an "incomplete" pedigree. It is, of course, incomplete in that we have no record as to whether a grandparent was born single, in twins, or in triplets.

TABLES FOR SIRES, DAMS, AND OFFSPRING WITH A "COMPLETE AMERICAN" PEDIGREE

Table XVIII(A) corresponds to Table I(A). Here we find for the correlation between the sum of numbers in litters in which sire and dam are born and the number in corresponding litters in which offspring are born, $r = 0.129 \pm 0.011$.

This coefficient is greater than that for the total population by 0.041, and this difference is just about three times its probable error. Hence, it is doubtful whether the difference is significant.

[1] Wallace, A. R. Darwinism . . . p. 154. London, 1896.

TABLE XVIII(A).—*Correlation between offspring and pairs of parents with "complete American" pedigree*

Sires and dams.	Offspring.			Total.
	1	2	3	
1–1	825	368	8	1,201
1–2	562	357	6	925
2–1	493	282	1	776
2–2	338	275	13	626
1–3	3	11	.....	14
3–1	5	7	.....	12
2–3	6	8	3	17
3–2	3	3	3	9
3–3	.....	.....	.....	.....
Total	2,234	1,311	34	3,579

$$r = 0.129 \pm 0.011,$$

where r is the correlation coefficient between the sum of numbers in litters in which sire and dam are born and the number in corresponding litter in which offspring are born.

Means of arrays of offspring:

(1) When sire and dam are singles ..... 1.3197 ± 0.0094.
(2) When sire is a single and dam is a twin ..... 1.399 ± 0.011.
(3) When sire is a twin and dam is a single ..... 1.366 ± 0.012.
(4) When sire is a twin and dam is a twin ..... 1.481 ± 0.014.
(5) When either sire or dam is a triplet ..... 1.788 ± 0.058.

TABLE XVIII(B).—*Percentages of offspring in states 1, 2, and 3 to correspond to the states 1–1, 1–2, 2–1, . . . of the sires and dams*

Sires and dams.	Offspring.		
	1	2	3
1–1	68.69	30.64	0.67
1–2	60.75	38.59	.66
2–1	63.48	36.39	.13
2–2	53.99	43.93	2.08
(a)	32.69	55.77	11.54
Based on total	62.42	36.63	.95

a See footnote, Table I(B).

There is a slightly smaller proportion of twins among offspring of the "complete American" born than among the offspring of the total population, but the difference is only about three times its probable error, and there is some question as to its significance. The results obtained from means of arrays are not significantly different whether found from Tables I or XVIII.

TABLES FOR DAMS AND OFFSPRING WITH "COMPLETE AMERICAN" PEDIGREE

Table XIX(A) corresponds to Table II(A) but is limited to data from American-born grandparents. Here we have for the correlation of size of litter in which dams and offspring are born,

$$r = 0.128 \pm 0.010.$$

This coefficient is greater than that for the total population by 0.041, and this difference is slightly more than three times its probable error, but it still is far from certain that the difference is not to be attributed to random sampling.

TABLE XIX(A).—*Correlation between offspring and dams with "complete American" pedigree*

Dams.	Offspring.			Total.
	1	2	3	
1	1,505	709	9	2,223
2	985	704	24	1,713
3	10	21	3	34
Total	2,500	1,434	36	3,970

Means:
- Dams ..... 1.4486±0.0055.
- Offspring ..... 1.3793±0.0054.

Means of arrays:
- (1) When dam is a single ..... 1.3270±0.0068.
- (2) When dam is a twin ..... 1.4390±0.0082.
- (3) When dam is a triplet ..... 1.794 ±0.067.

$r = 0.128 \pm 0.010.$

TABLE XIX(B).—*Percentages of offspring in states 1, 2, and 3 to correspond to the states 1, 2, and 3 of the dams*

Dams.	Offspring.		
	1	2	3
1	67.70	31.89	0.41
2	57.50	41.10	1.40
3	29.41	61.76	8.83
Based on total	62.97	36.12	.91

TABLES FOR DAMS AND MATERNAL GRANDPARENTS WITH A "COMPLETE AMERICAN" PEDIGREE

Tables XX to XXV correspond to Tables III to VIII, and the results to be drawn from the tables based on data of the "complete American" pedigrees do not differ essentially from those drawn from the total population. Here again, as with the total population, there appears to be a slight correlation between size of litter in which maternal granddams and offspring are born, but we are unable to make a similar assertion about maternal grandsires and offspring.

TABLE XX(A).—*Correlation between offspring and maternal granddams with "complete American" pedigree*

Dams and maternal granddams.	Offspring.			Total.
	1	2	3	
1–1	946	432	6	1,384
1–2	543	271	3	817
2–1	539	362	9	901
2–2	459	333	15	798
1–3	16	6		22
3–1	4	6	2	12
2–3	5	9		14
3–2	5	10	1	16
3–3	1	5		6
Total	2,500	1,434	36	3,970

Means of arrays of offspring:

(1) When the dam and granddam are singles..... 1.3208±0.0086.
(2) When the dam is a single and the granddam a twin. 1.339 ±0.011.
(3) When the dam is a twin and the granddam a single. 1.412 ±0.011.
(4) When the dam is a twin and the granddam a twin.. 1.455 ±0.013.
(5) When either dam or granddam is a triplet..... 1.600 ±0.046.

TABLE XX(B).—*Percentages of offspring in states 1, 2, and 3 to correspond to the states 1–1, 1–2, 2–1, 2–2, ... of the dams and maternal granddams*

Dams and maternal granddams.	Offspring.		
	1	2	3
1–1	68.35	31.21	0.44
1–2	66.46	33.17	.37
2–1	58.82	40.18	1.00
2–2	56.39	41.73	1.88
(a)	44.28	51.43	4.29
Based on totals	62.97	36.12	.91

a See footnote, Table I (B).

TABLE XXI(A).—*Correlation between offspring and maternal grandsires with "complete American" pedigree*

Dams and maternal grandsires.	Offspring.			Total.
	1	2	3	
1–1	900	431	2	1,333
1–2	582	258	6	846
2–1	579	424	13	1,016
2–2	383	277	11	671
1–3	23	20	1	44
3–1	7	15	3	25
2–3	23	3	.....	26
3–2	3	6	.....	9
3–3	.....	.....	.....	.....
Total	2,500	1,434	36	3,970

TABLE XXI(B).—*Percentages of offspring in states 1, 2, and 3 to correspond to the states 1–1, 1–2, 2–1, ... of the dams and maternal grandsires*

Dams and maternal grandsires.	Offspring.		
	1	2	3
1–1	67.52	32.33	0.15
1–2	68.79	30.50	.71
2–1	56.99	41.73	1.28
2–2	57.08	41.28	1.64
(a)	53.85	42.31	3.84
Based on total	62.97	36.12	.91

[a] See footnote, Table I (B).

TABLE XXII.—*Correlation between offspring and maternal granddams with "complete American" pedigree*

Maternal granddams.	Offspring.			Total.
	1	2	3	
1	1,480	800	17	2,297
2	998	614	19	1,631
3	22	20	.....	42
Total	2,500	1,434	36	3,970

$r=0.040\pm0.011$.

Means of arrays of offspring:

(1) When maternal granddams are singles..... 1.3631±0.0070.
(2) When maternal granddams are twins..... 1.3898±0.0087.
(3) When maternal granddams are triplets..... 1.516 ±0.052.

TABLE XXIII.—*Correlation between offspring and maternal grandsires with "complete American" pedigree*

Maternal grandsires.	Offspring.			Total.
	1	2	3	
1	1,486	870	18	2,374
2	968	541	17	1,526
3	46	23	1	70
Total	2,500	1,434	36	3,970

$r = -0.0068 \pm 0.011$.

Means of arrays of offspring:

(1) When maternal grandsires are singles..... 1.3816 ± 0.0069.
(2) When maternal grandsires are twins..... 1.3768 ± 0.0090.
(3) When maternal grandsires are triplets..... 1.357 ± 0.041.

TABLE XXIV(A).—*Correlation between dams and maternal granddams with "complete American" pedigree*

Maternal granddams.	Dams.			Total.
	1	2	3	
1	1,175	725	11	1,911
2	673	627	11	1,311
3	18	11	4	33
Total	1,866	1,363	26	3,255

$r = 0.103 \pm 0.012$.

TABLE XXIV(B).—*Percentages of dams in states 1, 2, and 3 to correspond to states 1, 2, and 3 of the maternal granddams*

Maternal granddams.	Dams.		
	1	2	3
1	61.49	37.04	0.57
2	51.33	47.83	.84
3	54.55	33.33	12.12
Based on total	57.33	41.87	.80

TABLE XXV(A).—*Correlation between dams and maternal grandsires with "complete American" pedigree*

Maternal grandsires.	Dams.			Total.
	1	2	3	
1	1,111	808	19	1,938
2	718	531	7	1,256
3	37	24	.....	61
Total	1,866	1,363	26	3,255

$r=0.007\pm0.012$.

TABLE XXV(B).—*Percentages of dams in states 1, 2, and 3 to correspond to states 1, 2, and 3 of the grandsires*

Grandsires.	Dams.		
	1	2	3
1	57.33	41.69	0.98
2	57.17	42.28	.55
3	60.66	39.34	.....
Based on total	57.33	41.87	.80

For the "complete American" subclass (Table XXV) we are also unable to assert a significant correlation between maternal grandsire and dams. This fact makes it appear still a little more probable that sex is in some way connected with the tendency of twins to produce twins. [Compare Tables VIII(A) and XXV(A).] This point will be further treated under "Analysis of data on parents and offspring separated with regard to sex."

TABLES FOR SIRES AND OFFSPRING WITH A "COMPLETE AMERICAN" PEDIGREE

While from the data of Table XXVI(A) the correlation coefficient, $r=0.072\pm0.011$, is a little larger than for the total population, the difference may be ascribed to random sampling. Just as in the "Analysis of data for sires and offspring" we have found mean values of an index number for sires of different classes, so here we have the following values for mean index numbers for animals of "complete American" pedigree:

For sires born in singles ("complete American"), we have. 1.296±0.012.
For sires born in twins ("complete American"), we have. 1.375±0.017.
For remaining sires born in singles, we have..... 1.353±0.010.
For remaining sires born in twins, we have..... 1.384±0.014.

TABLE XXVI(A).—*Correlation between offspring and sires with "complete American" pedigree*

Sires.	Offspring.			Total.
	1	2	3	
1	1,390	736	15	2,141
2	836	565	17	1,418
3	8	10	2	20
Total	2,234	1,311	34	3,579

$r=0.072\pm0.011$

Means of arrays:

(1) When sires are singles ..... 1.3578±0.0072.
(2) When sires are twins ..... 1.4224±0.0093.
(3) When sires are triplets ..... 1.700 ±0.095.

TABLE XXVI(B).—*Percentages of offspring in states 1, 2, and 3 to correspond to the states 1, 2, and 3 of the sires with "complete American" pedigree*

Sires.	Offspring.		
	1	2	3
1	64.92	34.38	0.70
2	58.96	39.84	1.20
3	40.0	50.0	10.0
Based on total	62.42	36.63	.95

All our results with index numbers show consistently a larger relative production of twins by twin sires than by single sires. It may be noted, however, that in the case of the subclass remaining after those of "complete American" pedigree are removed, the difference is not three times its probable error. This surely means that it requires very large numbers to establish with any considerable certainty the significance of the difference.

TABLES FOR SIRES AND PATERNAL GRANDPARENTS

Tables XXVII to XXX for "complete American" pedigrees correspond to Tables X to XIII for the total population. Here again, as with the total population, we are unable to assert the existence of a significant correlation between paternal grandparents and offspring with respect to being born in singles or in twins.

TABLE XXVII(A).—*Correlation between offspring and paternal granddams with "complete American" pedigree*

Sires and paternal granddams.	Offspring.			Total.
	1	2	3	
1–1	750	415	8	1,173
1–2	619	303	6	928
2–1	401	297	11	709
2–2	421	265	6	692
1–3	21	19	1	41
3–1	7	5	2	14
2–3	14	2	.....	16
3–2	1	5	.....	6
3–3	.....	.....	.....	.....
Total	2,234	1,311	34	3,579

TABLE XXVII(B).—*Percentages of offspring in states 1, 2, and 3 to correspond to the states 1–1, 1–2, 2–1, . . . of the sires and paternal granddams with "complete American" pedigree*

Sires and paternal granddams.	Offspring.		
	1	2	3
1–1	63.94	35.38	0.68
1–2	66.70	32.65	.65
2–1	56.56	41.89	1.55
2–2	60.84	38.30	.86
(a)	55.84	40.26	3.89
Based on total	62.42	36.63	.95

[a] See footnote, Table I(B).

TABLE XXVIII.—*Correlation between offspring and paternal granddams with "complete American" pedigree*

Paternal granddams.	Offspring.			Total.
	1	2	3	
1	1,158	717	21	1,896
2	1,041	573	12	1,626
3	35	21	1	57
Total	2,234	1,311	34	3,579

$r = -0.028 \pm 0.011$.

TABLE XXIX(A).—*Correlation between offspring and paternal grandsires with "complete American" pedigree*

Sires and paternal grandsires.	Offspring.			Total.
	1	2	3	
1–1	869	466	5	1,340
1–2	520	270	10	800
2–1	459	342	6	798
2–2	369	216	8	593
1–3	1			1
3–1	8	6	2	16
2–3	17	7	3	27
3–2				
3–3		4		4
Total	2,234	1,311	34	3,579

TABLE XXIX(B).—*Percentage of offspring in states 1, 2, and 3 to correspond to the states 1–1, 1–2, 2–1, . . . of the sires and paternal grandsires with "complete American" pedigree*

Sires and paternal grandsires.	Offspring.		
	1	2	3
1–1	64.85	34.78	0.37
1–2	65.00	33.75	1.25
2–1	56.39	42.80	.75
2–2	62.23	36.42	1.35
(a)	54.17	35.42	10.41
Based on total	62.42	36.63	.95

[a] See note, Table I(B).

TABLE XXX.—*Correlation between offspring and paternal grandsires with "complete American" pedigree*

Paternal grandsires.	Offspring.			Total.
	1	2	3	
1	1,327	814	13	2,154
2	889	486	18	1,393
3	18	11	3	32
Total	2,234	1,311	34	3,579

$r = -0.0059 \pm 0.011$.

Tables XXXV and XXXVI exhibit data of the "complete American" class excluded under the criteria of "Repetition of sires and paternal grandparents."

ANALYSIS OF DATA ON PARENTS AND OFFSPRING SEPARATED WITH REGARD TO SEX

Table XXXI exhibits data for the correlation of size of litters in which sires are born and sizes of litters in which their female offspring are born. The importance of investigating the tendency of twin sires to produce a larger percentage of twins among female offspring than single sires produce among female offspring is suggested by the fact that, from the data of Tables VIII and XXV, we were unable to assert the existence of a significant correlation.

TABLE XXXI(A).—*Correlation between female offspring and sires*

Sires.	Female offspring.			Total.
	1	2	3	
1	2,373	1,388	11	3,772
2	1,440	930	19	2,389
3	15	27	1	43
Total	3,828	2,345	31	6,204

$r = 0.0410 \pm 0.0086$.

Means:

For sires ..... 1.3987 ± 0.0044.

For female offspring ..... 1.3880 ± 0.0043.

TABLE XXXI(B).—*Percentages of female offspring in states 1, 2, and 3 to correspond to states 1, 2, and 3 of the sires*

Sires.	Female offspring.		
	1	2	3
1	62.91	36.80	0.29
2	60.28	38.93	.79
3	34.88	62.79	2.33
Based on total	61.70	37.80	.50

From Table XXXI(A) we obtain for the correlation

$$r = 0.0410 \pm 0.0086.$$

While this is a very small correlation, it is 4.6 times its probable error. While it is by no means finally established, it seems somewhat more probable that the values in Tables VIII and XXV are accidentally small under random sampling than that the correlation just given is insignificant. What is clear is that if any correlation exists, such correlation is so small that it requires immense numbers to establish its significance.

In order to eliminate the possible effects of the repetitions of grandsires, we shall investigate further the cases of maternal grandsires and dams by means of index numbers, such as are explained in the sections on "Analysis of data for sires and offspring" and "Tables for sires and offspring." The results with such index numbers may be stated as follows:

For maternal grandsires born in singles, we have..... 1.4462±0.0073.
For maternal grandsires born in twins, we have..... 1.4574±0.0093.

While the mean index number for twin maternal grandsires is thus a little larger than for single maternal grandsires, the difference is not large enough to enable us to assert its significance, when compared to fluctuations in sampling.

Tables XXXII to XXXIV are correlation tables for sizes of litters in which sires and male offspring, dams and male offspring, and dams and female offspring are born. In each of these cases we find significant correlations. The differences of these correlations are so small that we are unable to assert that the differences are significant. The values of these correlation coefficients are:

For sires and male offspring..... $r=0.073\pm0.012$.
For dams and male offspring..... $r=0.075\pm0.011$.
For dams and female offspring..... $r=0.0925\pm0.0080$.

TABLE XXXII(A).—*Correlation between male offspring and sires*

Sires.	Male offspring.			Total.
	1	2	3	
1	1,099	663	14	1,776
2	724	549	18	1,291
3	7	12	1	20
Total	1,830	1,224	33	3,087

Means:
For sires..... 1.4310±0.0066.
For male offspring..... 1.4180±0.0067.

TABLE XXXII(B).—*Percentages of the male offspring in states 1, 2, and 3 to correspond to the states 1, 2, and 3 of the sires*

Sires.	Male offspring.		
	1	2	3
1	61.88	37.33	0.79
2	56.09	42.53	1.38
3	35.00	60.00	5.00
Based on total	59.28	39.65	1.07

TABLE XXXIII(A).—*Correlation between male offspring and dams*

Dams.	Male offspring.			Total.
	1	2	3	
1	1,195	704	12	1,911
2	861	657	19	1,537
3	26	20	3	49
Total	2,082	1,381	34	3,497

TABLE XXXIII(B).—*Percentages of male offspring in states 1, 2, and 3 to correspond to states 1, 2, and 3 of the dams*

Dams.	Male offspring.		
	1	2	3
1	62.53	36.84	0.63
2	56.02	42.75	1.23
3	53.06	40.82	6.12

TABLE XXXIV(A).—*Correlation between female offspring and dams*

Dams.	Female offspring.			Total.
	1	2	3	
1	2,589	1,342	15	3,946
2	1,725	1,273	21	3,019
3	32	38	.....	70
Total	4,346	2,653	36	7,035

TABLE XXXIV(B).—*Percentages of female offspring in states 1, 2, and 3 to correspond to states 1, 2, and 3 of the dams*

Dams.	Female offspring.		
	1	2	3
1	65.61	34.01	0.38
2	57.14	42.17	.69
3	45.71	54.29	.....
Based on total	61.78	37.71	.51

Tables XXXV and XXXVI exhibit data excluded, by the criteria of the section on "Repetition of sires and paternal grandparents," from the "complete American" subgroup.

TABLE XXXV.—*Sizes of litters in which offspring and pairs of parents are born, "complete American" (from data excluded in making Table XVIII)*

Sires and dams.	Offspring.			Total.
	1	2	3	
1–1	63			63
1–2	18			18
2–1	120	52		172
2–2	64	69	2	135
1–3				
3–1				
2–3	1	2		3
3–2				
3–3				
Total	266	123	2	391

TABLE XXXVI.—*Sizes of litters in which offspring and sires are born (from data excluded in making Table XXVI)*

Sires.	Offspring.			Total.
	1	2	3	
1	81			81
2	185	123	2	310
3				
Total	266	123	2	391

GENERAL CONCLUSIONS

(1) For the class of sheep considered in this investigation we find that, in general, the twin parents give a larger percentage of twins among offspring than do parents born as singles. See Tables I(B), II(B), VII(B), IX(B), and XIV(B).

(2) The small positive correlation coefficient between the sum of numbers in litters in which the two parents are born and the size of litter in which the corresponding offspring are born is significant. The value of the coefficient is in each case more than 11 times the probable error. See results derived from Tables I(A) and XVIII(A).

(3) The small positive correlation coefficients between sizes of litters in which dams are born and sizes of litters in which their offspring are born are decidedly significant when judged by probable errors. See

results derived from Tables II(A), VII(A), XXIV(A), XXXIII(A), and XXXIV(A).

(4) There appears to be a small but significant correlation between sizes of litters in which sires are born and sizes of litters in which their offspring are born. It seems probable that this correlation should be attributed almost entirely, if not wholly, to correlation between sires and male offspring. The correlations seem to differ with the sexes. The correlation coefficients for sires and female offspring are so small that their significance is much in doubt even with the large numbers we have used. See results derived from Tables VIII(A), IX(A), XXV(A), XXVI(A), XXXI(A), and XXXII(A).

(5) There appears to be a significant correlation between maternal granddams and offspring, but we are unable to assert any significant correlation for the other grandparents and offspring. It would surely require immense numbers to establish the significance of such correlation, if it exists.

(6) The means of arrays show the small but general tendency of either or both twin parents and twin maternal granddams to produce a larger proportion of twins than are produced when the corresponding individuals in the ancestry are singles.

(7) As it requires large numbers to establish the significance of the differences which we have found, it should not be surprising if within a flock of fair size, say, a flock of 100, one may in some cases get even a larger percentage of twins from single parents than from twin parents. Such fluctuations should be expected to occur occasionally in taking a random sample of no more than 100 individuals, even if in the long run twins tend to produce a somewhat higher percentage of twins than do singles.

SOIL PROTOZOA[1]

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I.—METHOD FOR COUNTING PROTOZOA

INTRODUCTION

Repeated claims that soil protozoa are detrimental to other soil microorganisms have led soil biologists to begin the study of soil protozoology in order to determine, if possible, to what extent these organisms influence soil fertility.[2]

In order to facilitate the counting and examination of protozoa, several investigators have cultivated these organisms in artificial culture solutions. Goodey (7) studied the protozoa which were developed in soil extract and dilute hay infusion, but made no attempt to count them. Likewise Martin and Lewin (13) made careful examinations of the organisms which they cultivated in an infusion of horse manure. Rahn (15) employed peptone and sugar solutions, thus allowing the organisms to develop in a culture solution of 1 to 100 dilution for from 7 to 14 days, after which an aliquot of the solution was examined for protozoa. Killer (10) used the dilution method in order to determine the approximate numbers of soil protozoa that were developed in Giltay's, mannite, and peptone solutions. Francé (6) studied soil protozoa developed in artificial culture solutions and enumerated the organisms from the various soils examined by mixing an average sample of the solution with water and then examining the resulting solution drop by drop. Likewise Cauda and Sangiorgi (2) employed Giltay's, Omelianski's, Hiltner's, peptone, and mannite solutions and determined the numbers of organisms developed by dilution and direct count. In order to find the best culture solution for protozoan development, Cunningham and Löhnis (4) grew these organisms in many different solutions, and later Cunningham employed soil extract and blood-meal extract. The last-named investigator (3) also used the dilution method for the enumeration of the organisms. The results obtained with the dilution method by these investigators have been somewhat irregular. The irregularity in the results secured by the application of this method is shown quite clearly by Cunningham (3). The same irregularity has been experienced by the writer, who found the error to be several hundred per cent in many cases.

[1] Contribution from the Laboratories of Biology, Soil Bacteriology, and Soil Chemistry of the New Jersey Agricultural College and Experiment Station.

[2] The writer wishes to take this opportunity to express his appreciation to Dr. J. G. Lipman for many suggestions and the information which he has supplied; likewise to Dr. F. E. Chidester for the services rendered throughout the study of this series of problems.

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IMPROVED LOOP METHOD

Because of the inadequacy of the methods heretofore used, the loop method which was employed by Müller (14) for counting bacteria was improved upon. The improved method proved much more accurate and required but a very small amount of manipulation.

A platinum wire was bent into a permanent loop. The quantity of solution that could be transferred by means of this loop was then determined by carefully weighing films of the culture solution on a sensitive analytical balance. The average of several weights was taken and the quantity of liquid that could be transferred by the loop was calculated into cubic centimeters. To facilitate the counting of the organisms on the slide, a quarter of an inch square in the center of an ordinary glass slide was carefully ruled into 60 to 80 small squares by means of a sharp quartz crystal. A film of the culture solution containing the living protozoa was then transferred to the ruled area on the clean glass slide. In this manner the living protozoa were counted with the low power of the microscope, and from the number of organisms transferred in the loop the numbers per cubic centimeter were calculated.

The platinum loop was slightly bent, making an angle of 30° to 35°, so as to facilitate touching the slide in the same manner at each transfer and to prevent the draining of the solution which would adhere to the support of the loop.

In making the counts the platinum loop was first sterilized in a flame, and every precaution ordinarily observed in bacteriological work was taken. The protozoa of not less than three loops of culture solution were counted, and the average of the several counts was recorded. It was found by experience that not more than 300 to 400 protozoa could be counted in a film which had been transferred by a loop of 0.0020 to 0.0025 gm. transference capacity, as the culture solution on the slide would be evaporated before all the organisms could be counted. Therefore, two platinum loops, one of 0.0020 to 0.0025 gm. transference capacity and the other of 0.001 gm., were employed. When the number of organisms became so great that they could not all be counted in the large film, the loop of smaller capacity was employed. Where the organisms numbered more than 300 for the small loop, the film of culture solution containing the protozoa was transferred to a specially prepared slide. This glass slide has a square cell in the center (3.7 by 3.7 mm.), the capacity of which is about 0.002 gm. of water at 22° C. (when the readings are made the cell is almost completely filled with culture solution, thus reducing the possibility of error due to capillarity). The surface within the cell is accurately ruled into 25 large divisions, and one of the large divisions again ruled into 25 small fields. The film of solution containing the organisms was carefully spread over the entire surface and a cover slip laid over the cell, thus preventing evaporation. The organisms

in several of the large fields were counted, and the average of these figures was multiplied by 25. The number obtained was then multiplied by the standard number of the platinum loop. With the average of several of such counts and calculations, the number of organisms was determined. In case the organisms were too small to be readily seen by means of the low power of the microscope and too numerous to be calculated from the large divisions of the slide, the approximate number could be obtained by counting the organisms in the smaller divisions.

When many very active flagellates were studied, the latter method of counting the organisms in several fields proved superior to the complete counting method, on account of the fact that the manipulator might count one organism several times. But, as a general rule, in every case where the organisms were not too active and where the organisms numbered from 300 to 400 in the solution transferred by the large loop and 300 by the small loop the direct count of all the organisms transferred by the loop was made, in order that the error incurred be the minimum.

In computing the organisms on the slide several fields in different positions were counted and averaged, in this way eliminating as much as possible the very small error which might be incurred, owing to the very slight capillarity.

The flagellates were always counted while active, as otherwise in many cases they could not be distinguished from cysts. In case of the ciliates, however, the organisms could be very easily distinguished when inactive. Thus, in counting the ciliates, the film of solution was first passed through the fumes of a 1 per cent osmic-acid solution, as suggested by Goodey (7). When many ciliates were present in a solution containing numerous flagellates, the counts of the living flagellates were made first, and then several other films of solution treated with osmic acid were examined and the number of ciliates determined.

In order to determine the variation in the amount of solutions which a platinum loop would transfer, two loops of different size prepared for this purpose were tested by weighing films of different culture solutions. The weights recorded below were made by placing the film of the solution upon a watch glass, which was counterpoised by another of the same size. The rider was placed at 0.1 mg. and the correct addition of weight calculated by oscillations. The temperature of the room was 22° C. and of the balance case 22.5° when the weights were made. (See Tables I–V.)

TABLE I.—*Variation in transference capacity of a platinum loop when used for several solutions and the variation after it had been used for a period of time* [a]

[Weight of solution transferred]

Distilled water at 22° C. when loop was first used.	Distilled water at 22° C. after loop used 45 days.	Distilled water at 5° C.	Hay infusion at 22° C.	Blood extract at 22° C.	Soil extract at 22° C.
Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
0.00200	0.00199	0.00150	0.00186	0.00194	0.00170
.00199	.00199	.00149	.00185	.00191	.00169
.00199	.00200	.00149	.00184	.00193	.00169
.00200	.00198	.00149	.00184	.00192	.00171
.00199	.00198	.00150	.00183	.00193	.00170
.00199	.00198	.00150	.00183	.00192	.....
[b].001993	[b].001987	[b].001445	[b].001841	[b].001925	[b].001698

[a] 1 gm. of distilled water at 22° C.=1 c. c. [b] Average.

TABLE II.—*Density of culture solutions compared with water at the same temperature*

Temperature.	Water.	Hay infusion.	Blood extract.
°C.			
6	0.999970	0.999040	0.998885
22	.997828	.997460	.997648

TABLE III.—*Experimental error when all the organisms of the loop numbering about 9,000 per cubic centimeter are counted under the low power of the microscope*

Number of films.	Number of organisms in film.	Deviation.	Deviation squared.
1	14	5.2	27.0
2	22	2.8	7.8
3	13	6.2	38.4
4	14	5.2	27.0
5	23	3.8	14.4
6	19	.2	.4
7	26	6.8	46.2
8	28	8.8	77.4
9	23	3.8	14.4
10	10	9.2	84.6
Sum	192.0	.....	337.6
Mean	19.2	.....	.....

$$\text{Probable error} = \pm 0.6745\sqrt{\frac{\Sigma D^2}{n(n-1)}}$$

$$= \pm 0.6745\sqrt{\frac{337.6}{90}} = 1.92.$$

$$= \pm 0.6745 \times 1.92 = 1.29.$$

Percentage of error = 1.29 ÷ 19.2 = 6.74.

TABLE IV.—*Experimental error when all the organisms of the loop numbering about 140,000 per cubic centimeter are counted under the low power of the microscope*

Number of films.	Number of organisms in film.	Deviation.	Deviation squared.
1	263	22.5	506.0
2	251	34.5	1,190.0
3	286	.5	.2
4	377	91.5	8,370.0
5	266	19.5	381.0
6	290	4.5	22.1
7	302	16.5	72.0
8	223	62.5	3,910.0
9	326	40.5	1,640.0
10	266	19.5	381.0
Sum	2,850	.....	16,672.3
Mean	285	.....	.....

$$\text{Probable error} = \pm 0.6745\sqrt{\frac{16,672.3}{90}} = 13.62.$$

$$= \pm 0.6745 \times 13.62 = 9.20.$$

$$\text{Percentage of error} = 9.20 \div 285.0 = 3.23.$$

TABLE V.—*Probable error when the organisms of several fields are counted and when the number totals about 450,000 per cubic centimeter*

Number of films.	Number of organisms.					Average.	Deviation.	Deviation squared.
	Field No. 1.	Field No. 2.	Field No. 3.	Field No. 4.	Field No. 5.			
1	56	30	24	30	22	32.4	4.2	17.6
2	40	10	35	31	23	27.8	8.8	77.5
3	20	15	18	25	23	16.2	20.4	416.0
4	23	24	28	25	32	26.4	10.2	104.0
5	26	37	29	53	45	38.0	1.4	1.9
6	36	48	53	51	62	50.0	13.4	179.8
7	26	31	32	26	19	26.8	9.8	96.0
8	33	52	42	60	69	51.2	14.6	213.2
9	61	64	57	52	49	56.6	20.0	400.0
10	35	36	59	31	44	41.0	4.4	19.3
Sum	.....	.....	.....	.....	.....	366.4	.....	1,525.3
Mean	.....	.....	.....	.....	.....	36.6	.....	.....

$$\text{Probable error} = \pm 0.6745\sqrt{\frac{1,525.3}{90}} = 4.15.$$

$$= \pm 0.6745 \times 4.15 = 2.799.$$

$$\text{Percentage of error} = 2.799 \div 36.6 = 7.64.$$

The average of the experimental error incurred in counting is therefore 5.87 per cent.

EFFICIENCY OF THE METHOD

Upon examining the foregoing data, it is seen that the variation in weights of successive amounts transferred from a solution at a given temperature is very slight, the greatest variation being not more than 1 per cent.

In applying the improved loop method all calculations are based upon distilled water at 22° C., or room temperature, as a standard. Hence, a correction must be made when solutions of different surface tension are employed, as the amount of culture solution transferred by the standard loop would vary with the liquid. This is noted in Table I in comparing the amount of distilled water, hay infusion, blood extract, and soil extract transferred with the same loop.

In order to facilitate calculations, 1 gm. of distilled water at 22° C. was used as the standard to represent 1 c. c.

Upon examining Table II, it is noted that the error incurred on account of the variation in the density of solutions used is practically negligible.

Where the number of organisms per cubic centimeter is relatively small, the experimental error incurred in counting all the organisms contained in the loop is 6.74 per cent, as seen from Table III. As shown in Table IV, when the number of organisms per cubic centimeter is greater and all the organisms in the loop are counted, the error is smaller. In counting relatively small numbers of organisms in several fields of a specially ruled slide the experimental error is greater in proportion than if the organisms of the entire loop are counted, as shown in Table V.

SUMMARY OF PART I

(1) While the improved loop method is by no means devoid of errors, it has proved much more satisfactory than any of the other above-mentioned methods.

(2) It makes possible the quantitative study of the development of organisms in solutions without greatly altering the culture solutions.

(3) It is comparatively simple and requires but little time for any single determination.

(4) The improved loop method requires additional calculation because corrections must be made when culture solutions of different surface tension than distilled water at 22° are employed, which is not the case when the volume is always constant.

(5) The average experimental error is about 7 per cent.

II. PROTOZOA OF GREENHOUSE SOILS

INTRODUCTION

That small organisms other than bacteria, fungi, algæ, and worms—i. e., protozoa—exist in rich soils was known by Ehrenberg (5) as far back as 1837. Greef (9) in 1866 recorded the presence of very large living

forms in the soil, but it was probably not until the work of Breal (1) in 1896, later by Francé (6), and by Wolff (19) in 1909, that mention was made of the probable effect which protozoa might have upon soil fertility due to the destructive influence which they might have upon other soil micro-organisms. Then again, the very extensive work on soil sterilization by Russell and Hutchinson (16, 17) in 1909 and 1913 has been carried out for the purpose of showing the effect of heat and antiseptics upon a detrimental factor in the soil, which those authors believe to be protozoa. In 1911 Goodey (7) describes the isolation of various protozoa from the soil and concludes that protozoa are inactive in normal soils. A few years later, however, he (8) concluded that the ciliated forms are present in the soil in the encysted condition and that the amœbæ and flagellates act as limiting factors. Martin (12), examining freshly collected soil for protozoa, concluded that the prevalence of protozoa in culture solutions was no indication of their presence in the living state in the soil. Upon the examination of cucumber-sick soil, Martin and Lewin (13) found eight different kinds of protozoa. Amœbæ were probably the dominant forms in the soil during examination. Flagellates were very rare. These investigators state that in absolutely saturated soils the ciliates may play an active part as a bacteria check but that "it is difficult to believe that they can exercise an important rôle in a sick soil-bed." Russell and Petherbridge (18) are also of the opinion that the factor which keeps down the numbers of bacteria in cucumber- and tomato-sick soil is biological. Killer (10), Rahn (15), Cunningham and Löhnis (4, p. 600), and later Cunningham (3) grew soil protozoa in various culture solutions and noted a great difference in the development of these organisms in artificial media.

Cunningham (3, p. 22) concludes that "the results given in this section prove conclusively that the soil protozoa, in solution at all events, exercise a very decided limiting effect on the numbers of bacteria."

DEVELOPMENT OF PROTOZOA IN ARTIFICIAL CULTURE SOLUTIONS

In this study it was the object of the writer:

(1) To compare the difference in numbers and species of protozoa developed in different culture solutions.

(2) To compare the protozoan development from varying amounts of soil.

(3) To compare the protozoan development from moist and dry soil.

(4) To compare the protozoan development from different greenhouse soils.

A large sample of clayey soil having a moisture content of 25 per cent and upon which alfalfa was grown in 1914 and then composted to a 20 per cent manure, was collected from a compost bin in the greenhouse. A portion of this soil was dried for three days at 35° C. Into 200 c. c.

Jena Erlenmeyer flasks were placed 100 c. c. portions of a 3 per cent dried-blood extract + 0.05 per cent of dibasic potassium phosphate (K_2HPO_4) prepared by boiling 30 gm. of dried blood with 1,000 c. c. of tap water for one hour and then adding 0.05 per cent of K_2HPO_4. In like manner 100 c. c. portions of Löhnis's (11, p. 118) soil extract with the addition of 0.05 per cent of dibasic potassium phosphate were put into another series of flasks. These flasks were plugged with cotton and sterilized in an autoclave and all the precautions taken as in bacteriological work. They were then carefully inoculated with 1, 2, 3, 5, 10, 20, 50, and 100 gm. portions of moist and dry soils. The solutions were examined under the microscope for living protozoa by carefully transferring a film of the culture solution to a clean glass slide. The inoculated flasks were then placed in a constant-temperature incubator and incubated at 22° C. Daily examinations at the same hour for a period of 30 days were made and the different types of protozoa enumerated by the improved loop method described on p. 512.

In order to compare the development of protozoa of different greenhouse soils, three other compost soils were collected:

(1) A 10 per cent manure and 10 per cent sand mixture upon which roses were grown in the greenhouse the previous year. At the time of collection this soil was exposed to the weather and contained 21.1 per cent of moisture.

(2) A 50 per cent manure mixture, which was also exposed to the weather and had a moisture content of 30.3 per cent. Roses were grown upon this soil the previous year.

(3) A 30 per cent compost which was planted to soy beans and corn seedlings. This was a dry soil, having a moisture content of 14.9 per cent.

Erlenmeyer flasks of 200 c. c. capacity containing 100 c. c. of the same extracts of dried blood and soil, as described above, were inoculated with 1, 20, 50, and 100 gm. portions of the four composts. These solutions were examined for protozoa and then incubated at 22° C. for eight days. At the same hour each day examinations and counts of the different types of protozoa were made.

The classification of the protozoa which was followed throughout this problem was as follows:

The small ciliates included all organisms from the smallest to and including *Colpidium colpoda* Ehrb. The vorticellæ were also included.

The large ciliates included all forms larger than *Colpidium colpoda*.

The flagellates included all the forms that were observed.

It was found at the outset that there were many minute organisms that could not be recognized easily under the low power, but which were easily seen with the high power of the microscope. Even under the high power it was impossible to distinguish between large bacteria and

what might appear to be protozoa. For this reason all of the protozoan counts were made under the low power. In order that the results might be concordant, every culture was examined about the same hour each day, for there is a great variation in the numbers of active organisms present at different hours during the day (Table VI). This is especially true for the first 10 to 15 days after inoculation.

TABLE VI.—*Variation per gram of soil in numbers of protozoa present in culture solutions at different hours of the same day*

Culture No.	Second day after inoculation.		Third day after inoculation.	
	9 a. m.	4 p. m.	9 a. m.	4 p. m.
721	3, 272, 000	5, 430, 000	16, 800, 000	9, 982, 000
722	6, 180, 000	7, 220, 000	6, 980, 000	5, 950, 000
723	505, 000	1, 117, 000	798, 000	610, 000
724	221, 400	585, 000	516, 000	221, 400

DEVELOPMENT OF PROTOZOA IN DIFFERENT CULTURE SOLUTIONS INOCULATED WITH VARYING AMOUNTS OF MOIST AND DRY SOIL

SMALL CILIATES.—The development of protozoa in the various solutions was quite different (as shown in Table VII). In comparing the development of the small ciliates in different media, it is apparent that the maximum development of these organisms occurred sooner in dried blood than in soil extract. In all inoculations of the dried-blood extract the greatest numbers were present on the third to the fourth day; the numbers then decreased, so that after the ninth day there were very few present throughout the remaining period of observation. In soil-extract solutions inoculated with the largest amounts of soil, the maximum development was from the third to the fourth day, whereas in solutions of the same extract inoculated with 1 gm. of dry soil they did not appear until the ninth day and on the sixteenth day with the same amount of moist soil. In both culture solutions the numbers of small ciliates developed were very small as compared with the number of flagellates. For the existence of small ciliates the soil extract seemed to be more favorable than dried blood. In comparing the numbers of organisms developed in the different solutions it was found that those which were inoculated with the largest amounts of soil did not show greater development than the ones to which the smallest amounts of soil had been added. Hence, on the gram basis more than a hundred times as many small ciliates developed from 1 gm. of soil as from 100 gm. It is noted that in nearly all inoculations of both media a greater number of organisms developed at an earlier period from the moist than from the dry soil. This difference was, however, not very great.

TABLE VII.—*Comparison of the numbers of small ciliates, large ciliates, and flagellates developed daily in different artificial culture solutions from varying amounts of moist and dry soil for a period of 30 days. These numbers are calculated to 1 gm. of dry soil*

Solution No.	Quantity of soil.	Kind of soil.	Medium.	Kind of organisms.	Days after inoculation.									
					1	2	3	4	5	6	7	8	9	10
	Gm.													
10	1	Moist	Blood extract	Small ciliates			82,500	618,000			165,000		82,500	
				Large ciliates					82,500	82,500	82,500		82,500	
				Flagellates		1,800,000	23,180,000	82,500						163,000
11	3	do	do	Small ciliates			53,600	134,000	26,800	26,800			26,800	
				Large ciliates	26,800		26,800	53,600						26,800
				Flagellates		684,000	853,000	53,600				413,000	412,000	253,000
12	5	do	do	Small ciliates			33,000	132,000		66,000	16,500			
				Large ciliates							16,500	33,000		
				Flagellates		115,200	372,600	33,000	49,500	16,500	165,000	229,000	132,000	99,000
13	10	do	do	Small ciliates			24,700			8,250	8,250	8,250	8,250	
				Large ciliates				8,250			8,250	8,250		
				Flagellates	8,250	100,000	118,400	16,500			16,500		49,500	41,200
14	20	do	do	Small ciliates			20,600				4,120		4,120	
				Large ciliates		8,250	4,120	4,120					4,120	
				Flagellates		166,000	37,120	16,500		20,600	28,800	8,250	12,400	24,700
15	50	do	do	Small ciliates			1,650	3,300				1,650		
				Large ciliates								1,650		
				Flagellates		115,200	37,000	1,650		3,300	4,950	4,950	4,950	6,600
16	100	do	do	Small ciliates	825		825					825		
				Large ciliates	825	825						825		
				Flagellates		134,000	5,970	2,470	825	1,650	2,470	3,300		
20	1	Dry	do	Small ciliates			61,800			309,000	127,000	61,800		
				Large ciliates				61,800	61,800	61,800		61,800	61,700	61,700
				Flagellates		447,000	704,000	1,063,000	258,000	136,500	61,800	247,300	185,000	432,000
21	3	do	do	Small ciliates			20,600	82,500						
				Large ciliates			20,600						26,000	26,000
				Flagellates		170,000	3,267,000	3,520,000	82,500	20,600	82,500	61,800		41,200
				Small ciliates			27,000		12,380	12,380	12,380	12,380		
22	5	do	do	Large ciliates		12,380				12,380			12,400	
				Flagellates		61,700	1,625,000	27,000	27,000	27,000		37,500		37,000
				Small ciliates			12,360					30,900		
23	10	do	do	Large ciliates			6,180	6,180	6,180	6,180			6,180	
				Flagellates		18,530	307,000	12,360	6,180			18,530	12,360	12,360
				Small ciliates			6,180			3,090	3,090			
24	20	do	do	Large ciliates										
				Flagellates		12,360	123,600	101,970	3,090	3,090	3,090	30,900	3,090	18,540
				Small ciliates			2,480	1,240	2,480	1,240	1,240	1,240	1,240	
25	50	do	do	Large ciliates										
				Flagellates		18,530	13,400	33,000		1,240	1,240	3,720		3,700

26	100	...do...	...do...	Small ciliates...						618			618	
				Large ciliates...		618								
				Flagellates...		57,600	24,720	25,700		1,860		1,270	618	618
30	1	Moist...	Soil extract...	Small ciliates...										
				Large ciliates...										
				Flagellates...									183,000	3,900,000
31	3	...do...	...do...	Small ciliates...				29,800				29,800		
				Large ciliates...								29,800		
				Flagellates...						29,800	204,500	61,000	206,000	124,000
32	5	...do...	...do...	Small ciliates...								30,700	55,000	18,300
				Large ciliates...					18,350	18,350	18,350			
				Flagellates...				53,200	1,479,000	688,000	183,500	73,500		18,300
33	10	...do...	...do...	Small ciliates...				9,170	9,170	9,170	9,170	9,170	18,300	9,150
				Large ciliates...									18,300	
				Flagellates...			27,500	705,000	1,238,000	36,700	55,000	27,500	18,300	45,800
34	20	...do...	...do...	Small ciliates...				18,300	4,570			9,170		
				Large ciliates...		4,580		4,570	4,570	4,570	4,570	4,570	4,570	
				Flagellates...			51,600	157,200		4,570	13,700		18,300	13,750
35	50	...do...	...do...	Small ciliates...			3,670	3,670	1,830	5,500	1,830			
				Large ciliates...			1,830	1,830	1,830			1,830	1,830	
				Flagellates...		12,600	57,300	5,500	1,830	3,670	9,180	1,830	19,500	10,400
				Small ciliates...			3,670	918	918	918	918	918	918	
36	100	...do...	...do...	Large ciliates...		1,830	918	918				2,800	918	
				Flagellates...		24,020	20,620	918	2,860	2,860	2,860	2,860	6,420	9,160
40	1	Dry...	...do...	Small ciliates...									68,500	
				Large ciliates...										
				Flagellates...										
41	3	...do...	...do...	Small ciliates...						45,700	68,700		22,900	
				Large ciliates...								22,900	45,700	
				Flagellates...					271,500	359,000	6,800,000	805,000		45,700
42	5	...do...	...do...	Small ciliates...				13,750	13,750	13,750	13,750	13,750	13,700	13,700
				Large ciliates...									13,700	
				Flagellates...				515,000	1,560,000	161,000	945,000	41,300		13,700
43	10	...do...	...do...	Small ciliates...				6,870	6,870	6,870	13,750			6,870
				Large ciliates...							6,870			6,870
				Flagellates...			71,200	1,105,700	686,000	55,000	75,000	62,000	13,700	13,700
44	20	...do...	...do...	Small ciliates...					3,440				6,870	
				Large ciliates...										
				Flagellates...		10,320	160,000	250,000	184,000	6,870	44,700	6,820	3,435	218,000
				Small ciliates...				1,375					2,740	1,370
45	50	...do...	...do...	Large ciliates...			1,375		1,375				1,370	
				Flagellates...		17,200	20,620	4,130	4,130	4,130		4,130		2,740
				Small ciliates...				687	687		687	687		
46	100	...do...	...do...	Large ciliates...			687							
				Flagellates...		50,350	38,630	2,000	1,375		687		687	1,370

TABLE VII.—*Comparison of the numbers of small ciliates, large ciliates, and flagellates developed daily in different artificial culture solutions from varying amounts of moist and dry soil for a period of 30 days. These numbers are calculated to 1 gm. of dry soil*—Continued

Solution No.	Quantity of soil.	Kind of soil.	Medium.	Kind of organisms.	Days after inoculation.									
					11	12	13	14	15	16	17	18	19	20
	Gm.													
10	1	Moist	Blood extract	Small ciliates			82,500	82,500	82,500			82,500	82,500	
				Large ciliates		82,500								
				Flagellates					247,500	82,500	82,500	82,500	82,500	103,000
				Small ciliates									26,800	
11	3	do	do	Large ciliates										
				Flagellates	824,000	218,000	53,600	164,000	26,800	257,000	26,800			53,600
				Small ciliates										
12	5	do	do	Large ciliates					16,100					
				Flagellates	66,000	247,000	330,000	299,000	330,000	165,000	66,000	181,000	66,000	692,000
				Small ciliates					8,250			8,250		
13	10	do	do	Large ciliates						8,250				
				Flagellates	8,250	8,250	385,000	16,600	16,600	66,000	41,200	49,500	24,700	107,000
				Small ciliates	4,120	4,120		4,120				4,120	4,120	
14	20	do	do	Large ciliates										
				Flagellates	24,700	28,800	28,800	45,400	57,700	24,700	4,120	16,500	12,400	8,250
				Small ciliates					1,660			1,660	3,300	1,660
15	50	do	do	Large ciliates			1,660			1,660				
				Flagellates	8,250	13,200	13,200	3,300	4,950	3,300	1,660	4,950	6,600	6,600
				Small ciliates			825	825				825	825	
16	100	do	do	Large ciliates										
				Flagellates	5,760	2,470	4,950	825	1,650	2,470	825	3,300	1,650	4,120
				Small ciliates					61,700	61,700	61,700			61,700
20	1	Dry	do	Large ciliates								61,700		
				Flagellates	61,700	61,700	123,500			123,500	61,700			61,700
				Small ciliates									26,000	
21	3	do	do	Large ciliates	26,000									82,500
				Flagellates		144,000	166,000		166,000	41,200	41,200	477,000	41,200	12,400
				Small ciliates										12,400
22	5	do	do	Large ciliates										37,000
				Flagellates		37,000	49,500		144,000	12,400	37,000	49,500	24,700	
				Small ciliates	6,180				6,180			6,180	12,360	
23	10	do	do	Large ciliates										6,180
				Flagellates	12,360	12,360	12,360		10,300	6,180		12,360	6,180	
				Small ciliates			6,180	6,180		3,090	9,270	3,090	6,180	
24	20	do	do	Large ciliates										
				Flagellates	3,090	6,180		6,180	6,180	3,090	9,270	6,180	3,090	3,090

25	50	do.	do.	Small ciliates	.....	.....	1,240	4,950	.....	2,480	3,700	6,180	1,248	3,700
				Large ciliates	.....		.....	.....	.....	.....	.....	.....	1,240	.....
				Flagellates	.....	1,240	.....	.....	2,480	.....	2,480	.....	2,480	.....
26	100	do.	do.	Small ciliates	618	618	1,230	618	618	618	618	3,090	618	618
				Large ciliates	.....	.....	.....	618	.....	.....	.....	618	.....	.....
				Flagellates	618	.....	618	.....	.....	618	.....	618	.....	618
30	1	Moist	Soil extract	Small ciliates	.....	.....	.....	.....	.....	183,000	91,500	183,000	.....	91,500
				Large ciliates	.....	.....	.....	.....	.....	91,500	.....	.....	.....	91,500
				Flagellates	53,700,000	46,700,000	25,000,000	19,600,000	11,625,000	6,470,000	9,900,000	640,000	1,190,000	825,000
31	3	do.	do.	Small ciliates	29,800	.....	.....	.....	.....	29,800	.....	29,800	29,800	29,800
				Large ciliates	.....	.....	29,800	.....	.....	29,800	.....	.....	.....	.....
				Flagellates	149,000	59,500	59,500	29,800	470,000	179,000	6,150,000	119,000	59,500	29,800
32	5	do.	do.	Small ciliates	36,700	36,700	18,300	.....	18,300	.....	.....	36,700	18,300	.....
				Large ciliates	18,300	.....	18,300	.....	18,300	.....	.....	.....	.....	18,300
				Flagellates	36,700	.....	.....	36,700	18,300	18,300	.....	.....	18,300	18,300
33	10	do.	do.	Small ciliates	36,700	9,150	18,350	18,350	9,150	9,150	9,150	36,800	9,150	9,150
				Large ciliates	.....	9,150	.....	.....	.....	.....	.....	.....	.....	.....
				Flagellates	82,500	91,500	137,000	146,500	45,700	9,150	18,350	18,350	27,500	27,500
34	20	do.	do.	Small ciliates	4,570	4,570	.....	.....	.....	.....	.....	.....	4,570	.....
				Large ciliates	.....	4,570	4,570	.....	.....	.....	.....	.....	.....	.....
				Flagellates	9,150	4,570	.....	9,150	41,300	9,150	18,350	59,500	105,520	13,700
35	50	do.	do.	Small ciliates	.....	.....	.....	.....	3,260	1,835	1,835	1,835	1,835	.....
				Large ciliates	.....	.....	.....	.....	.....	.....	1,835	.....	.....	.....
				Flagellates	3,670	12,600	16,000	10,320	13,700	5,500	7,300	7,300	3,660	5,500
36	100	do.	do.	Small ciliates	918	.....	.....	.....	.....	1,835	918	918	3,660	2,750
				Large ciliates	.....	918	.....	918	918	.....	.....	.....	.....	918
				Flagellates	4,590	8,250	2,750	9,180	9,180	7,300	8,750	918	2,750	1,830
40	1	Dry	do.	Small ciliates	68,500	68,500	.....	68,500	.....	68,500	68,500	68,500	68,500	68,500
				Large ciliates	.....	.....	.....	.....	.....	68,500	.....	.....	68,500	68,500
				Flagellates	137,500	890,500	5,270,000	12,300,000	29,100,000	12,000,000	10,140,000	200,000	68,500	.....
41	3	do.	do.	Small ciliates	.....	.....	.....	.....	.....	.....	.....	45,700	45,700	22,900
				Large ciliates	22,900	.....	.....	.....	.....	.....	.....	.....	22,900	.....
				Flagellates	22,900	367,000	480,000	92,000	72,000	69,500	620,000	22,900	92,000	92,000
42	5	do.	do.	Small ciliates	.....	.....	.....	.....	.....	.....	.....	.....	13,700	.....
				Large ciliates	.....	13,700	.....	.....	.....	13,700	.....	.....	.....	.....
				Flagellates	54,800	413,000	149,000	82,500	298,000	41,300	235,000	68,700	27,400	27,400
43	10	do.	do.	Small ciliates	6,870	6,870	6,870	13,700	6,870	6,870	.....	6,870	6,870	.....
				Large ciliates	.....	.....	.....	.....	.....	.....	.....	.....	.....	6,870
				Flagellates	27,400	61,700	13,700	13,700	20,600	6,870	13,700	6,870	13,700	.....
44	20	do.	do.	Small ciliates	13,700	3,440	.....	3,440	3,440	3,440	3,440	6,950	3,440	3,440
				Large ciliates	.....	.....	.....	.....	3,440	.....	.....	.....	.....	.....
				Flagellates	39,000	34,400	20,600	6,870	13,700	3,440	10,320	13,700	6,950	.....
45	50	do.	do.	Small ciliates	4,130	.....	1,370	1,370	1,370	1,370	1,370	.....	2,740	2,740
				Large ciliates	.....		.....	1,370	.....	1,370	.....	.....	.....	.....
				Flagellates	2,740	10,940	9,620	68,700	9,130	.....	.....	1,370	2,740	.....
46	100	do.	do.	Small ciliates	687	.....	.....	687	687	1,370	1,370	687	687	687
				Large ciliates	.....	.....	687	.....	.....	.....	.....	.....	687	.....
				Flagellates	687	.....	1,370	687	2,060	687	1,370	.....	687	2,060

TABLE VII.—*Comparison of the numbers of small ciliates, large ciliates, and flagellates developed daily in different artificial culture solutions from varying amounts of moist and dry soil for a period of 30 days. These numbers are calculated to 1 gm. of dry soil*—Continued

Solution No.	Quantity of soil.	Kind of soil.	Medium.	Kind of organisms.	Days after inoculation.									
					21	22	23	24	25	26	27	28	29	30
	Gm.													
10	1	Moist	Blood extract	Small ciliates	82,500	.....	.....	82,500	165,000	.....	330,000	165,000	247,000	165,000
				Large ciliates	82,500	.....	.....	.....	.....	.....	82,500	.....	.....	.....
				Flagellates	165,000	165,000	165,000	165,000	330,000	82,500	165,000	82,500	165,000	.....
11	3	...do	...do	Small ciliates	.....	.....	26,800	.....	53,600	26,800	53,600	26,800	.....	26,800
				Large ciliates	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
				Flagellates	26,800	80,400	53,600	26,800	26,800	.....	53,600	80,400	80,400	640,000
12	5	...do	...do	Small ciliates	.....	99,000	16,600	16,600	.....	16,600	33,200	99,000	16,600	16,600
				Large ciliates	.....	16,600	.....	.....	.....	.....	.....	.....	.....	.....
				Flagellates	99,000	33,200	99,000	115,000	181,000	82,500	165,000	82,500	99,000	198,000
13	10	...do	...do	Small ciliates	8,250	.....	.....	8,250	.....	8,250	.....	8,250	.....	.....
				Large ciliates	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
				Flagellates	24,700	107,000	16,600	82,500	90,700	66,000	82,500	41,250	49,500	99,000
14	20	...do	...do	Small ciliates	.....	8,250	4,125	4,125	8,250	.....	4,125	.....	4,125	.....
				Large ciliates	.....	.....	.....	.....	.....	.....	.....	.....	.....	4,125
				Flagellates	116,400	107,000	16,500	20,600	4,125	8,250	16,500	4,125	33,000	53,600
15	50	...do	...do	Small ciliates	.....	4,940	1,650	1,650	.....	1,650	1,650	1,650	4,940	1,650
				Large ciliates	1,650	1,650	.....	3,300	.....	.....	.....	.....	.....	.....
				Flagellates	1,650	1,650	.....	1,650	1,650	1,650	1,650	1,650	.....	1,650
16	100	...do	...do	Small ciliates	1,650	.....	1,650	825	.....	.....	825	.....	825	825
				Large ciliates	.....	825	.....	825	.....	.....	.....	.....	.....	.....
				Flagellates	1,650	4,125	5,775	5,775	1,650	825	1,650	825	825	1,650
20	1	Dry	...do	Small ciliates	.....	.....	61,800	123,800	61,800	.....	.....	.....	.....	.....
				Large ciliates	.....	.....	61,800	61,800	.....	61,800	.....	.....	.....	.....
				Flagellates	.....	61,800	61,800	61,800	.....	123,800	556,000	495,000	1,122,000	3,800,000
21	3	...do	...do	Small ciliates	.....	.....	.....	.....	.....	20,600	.....	.....	.....	.....
				Large ciliates	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
				Flagellates	20,600	206,000	61,800	144,000	144,000	61,800	41,200	103,000	123,500	144,000
22	5	...do	...do	Small ciliates	.....	12,350	.....	12,350	.....	.....	12,350	.....	.....	.....
				Large ciliates	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
				Flagellates	12,350	37,050	.....	24,700	12,350	49,500	274,000	149,000	262,000	257,000
23	10	...do	...do	Small ciliates	.....	.....	.....	.....	.....	.....	.....	.....	6,180	.....
				Large ciliates	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
				Flagellates	6,180	18,540	12,360	24,700	12,360	18,540	12,360	6,180	12,360	12,360
24	20	...do	...do	Small ciliates	6,180	6,180	6,180	3,090	6,180	3,090	9,270	3,090	.....	.....
				Large ciliates	3,090	.....	.....	.....	3,090	.....	.....	.....	.....	.....
				Flagellates	6,180	12,360	6,180	9,270	6,180	.....	6,180	3,090	3,090	6,180

25	50	...do...	...do...	Small ciliates	3,700	3,700	3,700	1,240	1,240		1,240	1,240			
				Large ciliates						1,240				1,240	
				Flagellates				1,240			1,240			618	618
				Small ciliates				1,230	2,460	3,090	618	618	618	618	
26	100	...do...	...do...	Large ciliates		618	618	618							
				Flagellates	1,230	1,230	618	618	1,230	1,850	3,090	2,470	1,850	4,950	
30	1	Moist...	Soil extract...	Small ciliates	92,000	183,000	183,000	183,000	92,000	92,000	275,000	275,000	183,000	92,060	
				Large ciliates	92,000	92,000				92,000	92,000		92,000		
				Flagellates	183,000	92,000									
31	3	...do...	...do...	Small ciliates	29,800		89,300	29,800							
				Large ciliates			59,600					29,800			
				Flagellates	29,800	59,600			59,600	29,800		29,800			
				Small ciliates			4,570				36,600	55,000	36,600		
32	5	...do...	...do...	Large ciliates											
				Flagellates	18,300	6,400	13,700	36,600	36,600	18,300		73,500	36,600	55,000	
				Small ciliates	9,200		18,300	9,200	9,200		18,300	9,200	9,200	9,200	
33	10	...do...	...do...	Large ciliates									9,200		
				Flagellates	9,200		18,300	18,300	18,300	9,200	9,200	18,300	9,200	18,300	
				Small ciliates			9,200	4,570	4,570	13,700	4,570				
34	20	...do...	...do...	Large ciliates											
				Flagellates	9,200	18,300		13,700	9,200	9,200	13,700	37,000	18,320	9,200	
				Small ciliates	1,830		1,830	1,830	1,830			1,830			
35	50	...do...	...do...	Large ciliates											
				Flagellates	3,670	13,700	1,370	49,300	63,000	28,700	18,310	50,500	27,350	88,300	
				Small ciliates	2,740	2,740	1,830	1,830	2,740	918	1,830	1,830	1,830	2,740	
36	100	...do...	...do...	Large ciliates		618			918		918				
				Flagellates	918	1,830		918	918			2,740	918		
40	1	Dry...	...do...	Small ciliates	137,500	137,500	68,700	68,700	68,700	68,700	68,700	68,700	68,700	68,700	68,700
				Large ciliates	68,700	68,700	68,700	68,700	68,700	137,500	68,700	275,000	68,700	413,000	
				Flagellates		68,700	620,000	137,500	137,500					22,900	
41	3	...do...	...do...	Small ciliates	22,900				22,900					22,900	
				Large ciliates			22,900								
				Flagellates	22,900	45,800	68,700	68,700	45,800		22,900	22,900			
42	5	...do...	...do...	Small ciliates		13,700	13,700								
				Large ciliates		13,700									
				Flagellates	13,700	27,400	96,000	27,400	27,400	41,100	13,700	27,400	13,700	27,400	
43	10	...do...	...do...	Small ciliates	6,870	1,370	6,870	20,600	6,870	6,870	6,870	6,870	6,870		
				Large ciliates				6,870			6,870				
				Flagellates	1,370	34,400	1,370	6,870	13,700	20,600	13,700	13,700			
44	20	...do...	...do...	Small ciliates	3,440		6,870	3,440	3,440		3,440	3,440			
				Large ciliates											
				Flagellates	3,440	6,870	6,870	3,440			3,440	3,440	3,440	3,440	
45	50	...do...	...do...	Small ciliates	1,370	2,740	2,740	2,740	4,130	1,370		2,740	1,370	1,370	
				Large ciliates			1,370								
				Flagellates	1,370	2,740	1,370	1,370	2,740	1,370	1,370	1,370	1,370	12,400	
				Small ciliates	687	1,370	2,750	687	1,370		2,060	687	687		
46	100	...do...	...do...	Large ciliates					687						
				Flagellates	687	5,500	4,800	4,130	6,170	2,060	1,370	2,750	687	4,130	

LARGE CILIATES.—Very few large ciliates developed in any of the inoculated solutions. These organisms appeared on the first day in some moist soil inoculations of dried-blood extract. In blood extract to which dry soil had been added and soil extract inoculated with moist soil large ciliates appeared on the second day in some solutions and on the third day in soil extract containing the largest amounts of dry soil. In all inoculations of dried-blood extract the greatest numbers appeared from the third to the eighth day; then the numbers decreased, so that there were very few in any of the solutions after the ninth day. In soil extract the organisms appeared much sooner in solutions with the larger than in the solutions with the smaller soil inoculations. As noted in Table VII, in solution 36, with 100 gm. of moist soil, the organisms developed on the second day, while in solution 30, when 1 gm. was used, the organisms did not appear until the nineteenth day. As many large ciliates developed in the dried blood as in soil extract. Again there was no definite relation between the numbers of organisms developed and the quantities of soil used for inoculation.

FLAGELLATES.—As noted in Table VII, in all inoculations of dried-blood extract, the maximum number of flagellates were present at an earlier period than in inoculations of soil extract. The maximum development in dried blood was from the second to the fourth day, depending on the quantity and kind of soil used. The largest number of organisms appeared sooner in solutions inoculated with the largest amounts of soil than where small quantities were used. With 100-gm. inoculations the maximum development of flagellates in solutions of soil extract with both moist and dry soils was on the seventh day, while with 1 gm. of moist soil the greatest numbers of organisms appeared on the eleventh day, and on the fifteenth day with the same amount of dry soil. As soon as the maximum numbers were reached, there was a gradual decrease until but few organisms remained in the solutions. In culture solutions inoculated with moist and dry soils, the largest development of flagellates was reached in the soil extract with the smallest quantities of soil, while with the largest amounts of soil the greatest development was in dried blood. In soil extract, with one exception, a larger number of flagellates were developed from the dry than from moist soil; this, however, was not the case with inoculations in blood. In nearly all inoculations with dry soil, the greatest numbers were developed in soil extract. In all cases there were nearly 200 times as many organisms developed per gram, from the 1-gm. than from the 100-gm. inoculations. In inoculated solutions of blood extract, the maximum number of all organisms appeared between the third and fourth day, while in the soil extract the largest number were present from the second to the fifteenth day, depending upon the kind and amount of soil inoculated.

DEVELOPMENT OF PROTOZOA FROM DIFFERENT COMPOST SOILS

As shown in Table VIII, the development of small ciliates, with respect to amounts of soil and media, was practically identical with the development in moist soil, as shown in Table VII. It is apparent from Table VIII that more small and more large ciliates develop from the less composted soils. In all inoculations of dried blood with the 10 and 20 per cent composts and for the larger amounts inoculated from the 30 and 50 per cent composts the maximum number of flagellates were present from the sixtieth hour to the fourth day. But with smaller amounts of soil inoculations from the 30 and 50 per cent manure soils, the maximum numbers appeared from the fourth to the seventh day. In the case of dried blood there was little difference in the numbers of flagellates developed from the different amounts of soil, while in soil extract inoculated with the smallest quantities of soil the maximum had not yet been reached at the end of the eighth day; hence, no comparison could be made. From the 30 and 50 per cent composts more flagellates were developed in blood extract than from the 10 and 20 per cent manures. Considering the numbers of organisms developed, on the gram basis, with dried blood there were more than two hundred times as many flagellates developed from 1 gm. as compared with 100 gm. of soil. It is seen that the maximum number of all organisms developed from the sixtieth hour to seven days in dried blood, while in the case of soil extract the maximum numbers had not been reached for the smaller soil inoculation when the experiment was concluded.

DEVELOPMENT OF DIFFERENT TYPES OF PROTOZOA FROM THE SOIL

Very many different types of large ciliates were developed. Of the forms identified *Paramecium* sp. was probably the most common. Other large ciliates which were noted were probably *Encheyls pupa* Ehrb., a few individuals of *Urolaptus musculus* Ehrb., and probably *Nassula elegans* Ehrb. The vorticellæ were very numerous, appearing the fourth day, and were present in some solutions throughout the period of 30 days. They were developed in dried blood and soil extract from both moist and dry soils. Next in prominence was the *Colpoda cucullus* O. F. M., which was first recognized on the fourth day. *Colpidium colpoda* Ehrb. was also quite common. Of the flagellates species resembling *Monas guttula* Ehrb. and *Monas vivipara* Ehrb. were the most common. *Bodos* spp. were also very common. A few dimastigate amœbæ, apparently *Amœba radiata* Kelbs, were seen between the fifteenth and twentieth day after inoculation. A species corresponding to *Peranema trichophorum* Ehrb. appeared in small numbers from the eighteenth to the twenty-second day. Likewise a few organisms resembling *Trinema anchelys* Ehrb. were observed. Very few amœbæ

TABLE VIII.—*Comparison of the numbers of small ciliates, large ciliates, and flagellates developed daily in different artificial culture solutions and from varying amounts of different greenhouse soils for a period of 8 days. These numbers are all calculated to 1 gm. of dry soil*

Solution No.	Quantity of soil.	Kind of soil.	Medium.	Kind of organisms.	Period after inoculation.							
					12 hours.	36 hours.	60 hours.	4th day.	5th day.	6th day.	7th day.	8th day.
	Gm.											
201	1	10 per cent compost	Soil extract	Small ciliates						87,300		
				Large ciliates						87,300		
				Flagellates				172,000				
202	20	do	do	Small ciliates			4,350	22,300	4,350	4,350	4,350	
				Large ciliates								4,350
				Flagellates			4,350	91,500	56,000	87,000	4,350	
203	50	do	do	Small ciliates			1,720	3,440	1,720			1,730
				Large ciliates								3,440
				Flagellates			42,300	613,000	50,500	3,440	5,160	
				Small ciliates				7,180	870	870	870	870
204	100	do	do	Large ciliates								
				Flagellates		7,900	84,600	221,600	1,720	1,720	870	4,350
				Small ciliates			78,300	1,590,000	78,300	79,300		79,300
211	1	do	Blood extract	Large ciliates				78,300	158,800			
				Flagellates		235,000	8,520,000	32,200,000	2,680,000	2,180,000	1,340,000	2,180,000
				Small ciliates			3,920	3,920	3,920		7,830	
212	20	do	do	Large ciliates								
				Flagellates	3,920	15,650	37,000	58,700	7,850	15,625		
				Small ciliates			1,550	1,550	1,550	1,550	1,550	
213	50	do	do	Large ciliates							1,550	
				Flagellates	1,540	3,045	288,000	212,000	12,350	9,370	3,090	3,090
				Small ciliates			785			785	785	1,550
214	100	do	do	Large ciliates								
				Flagellates	3,045	7,820	168,000	52,500	5,150		785	3,090
				Small ciliates						91,500		91,500
101	1	20 per cent compost	Soil extract	Large ciliates								
				Flagellates					367,000			
				Small ciliates				9,150		4,570	4,570	4,570
102	20	do	do	Large ciliates			4,570	4,570				
				Flagellates			9,150	745,000	662,000	497,000	27,500	55,000
				Small ciliates				3,670			3,670	
103	50	do	do	Large ciliates								
				Flagellates	1,830	1,830	3,850,000	6,730,000	62,000	33,200	9,150	18,300
				Small ciliates			697	1,100		697	697	4,130
104	100	do	do	Large ciliates								
				Flagellates		3,440	149,700	172,000	2,750	9,650	7,550	25,200

111	1	do	Small ciliates			82,500	165,000	165,000	330,000	82,500
			Flagellates			82,500	82,500	82,500	82,500	82,500
			Small ciliates		3,960,000	49,800,000	80,600,000	660,000	247,000	165,000
			Large ciliates			8,250	247,000			247,000	4,120	4,120
112	20	do	Flagellates	4,120	241,000	165,000	61,800	8,250	4,120	165,000
			Small ciliates		1,650	3,300	9,880	247,000	24,700	1,650	1,650	3,300
113	50	do	Large ciliates	1,650	63,800	230,500	171,000	24,700	6,600	132,000	19,800
			Flagellates			825	3,300					
114	100	do	Small ciliates			825	32,500	4,950	1,650	4,120	8,250
			Large ciliates	8,240	49,500							82,500
			Flagellates				80,300			82,500	82,500
401	1	30 per cent compost	Small ciliates					4,950	132,000	4,950	8,000
			Large ciliates									
402	20	do	Small ciliates					498,000	16,000	4,950	261,000
			Large ciliates							148,000	1,600
			Flagellates		64,700	1,650,000	3,120	1,600	17,600	12,300
403	50	do	Small ciliates		4,620	4,620		48,200	3,200	1,600
			Large ciliates		1,600	11,170	431,500	1,600	800		
			Flagellates			800	440,000				
404	100	do	Small ciliates					80,300	2,400	4,620
			Large ciliates	4,620	47,700	161,500	87,500				
			Flagellates								
411	1	Blood extract	Small ciliates					23,500,000	1,915,000	144,000	1,019,000
			Large ciliates			21,400,000	24,000,000		3,600	3,600	3,600
			Flagellates		217,000							
412	20	do	Small ciliates					171,000	129,000	7,200	32,400
			Large ciliates	3,600	173,000	171,000	172,000	2,590		1,440	1,440
			Flagellates		1,440		4,320				
413	50	do	Small ciliates		85,000	370,000	99,000	99,300	2,300	1,440	2,880
			Large ciliates	4,320	720			1,440	720		1,440
			Flagellates								
414	100	do	Small ciliates	2,880	9,370	41,800	7,200	7,200	1,440	720	1,440
			Large ciliates								
			Flagellates								
301	1	30 per cent compost	Small ciliates					98,500			
			Large ciliates								
			Flagellates			192,000		690,000			
302	20	do	Small ciliates					3,120,000	59,800		
			Large ciliates				4,930	4,930		4,930	4,930
			Flagellates								
303	50	do	Small ciliates								
			Large ciliates			365,000		590,000	51,500	33,200	551,000
			Flagellates			985	93,500				
304	100	do	Small ciliates								
			Large ciliates								
			Flagellates			985	233,000	169,000	4,930	19,800	38,800

TABLE VIII.—*Comparison of the numbers of small ciliates, large ciliates, and flagellates developed daily in different artificial culture solutions and from varying amounts of different greenhouse soils for a period of 8 days. These numbers are all calculated to 1 gm. of dry soil*—Continued

Solution No.	Quantity of soil.	Kind of soil.	Medium.	Kind of organisms.	Period after inoculation.							
					12 hours.	36 hours.	60 hours.	4th day.	5th day.	6th day.	7th day.	8th day.
	Gm.			Small ciliates		88,500						
311	1	50 per cent compost	Blood extract	Large ciliates								
				Flagellates		266,600	14,200,000	3,660,000	532,000	29,400,000	44,000,000	26,270,000
				Small ciliates		4,420	4,420					4,430
312	20	do	do	Large ciliates		4,420						
				Flagellates		17,700	149,500	1,570,000	87,500	39,200	22,200	26,600
				Small ciliates								
313	50	do	do	Large ciliates								
				Flagellates		7,120	422,000	299,000	37,100	3,500	5,250	8,850
				Small ciliates								
314	100	do	do	Large ciliates								
				Flagellates		885	17,800	241,000	16,800			885

were developed; one large form was developed from moist soil in dried-blood extract on the eighth day; thereafter no forms were recognized until the twenty-third and twenty-fourth day, when a few small forms were noted. Protozoan cysts were very numerous in the former part of the experiment, but they gradually disappeared until very few were seen after the twenty-second day, thus indicating that in culture solutions some of the protozoa do not encyst after they have once become active, but either die or are destroyed by other forms.

SUMMARY OF PART II

Under the conditions of the experiment and with the soils examined it was found that:

(1) In culture solutions the maximum development of small and large ciliates and flagellates varies with the culture solution and the condition and amounts of soil used for inoculation.

(2) In dried-blood extract the maximum development of all ciliates and flagellates is from the third to the fourth day, while in soil extract it is from the second to the fifteenth day, depending upon the character and amount of soil used for inoculation.

(3) When the maximum development of all organisms is reached, there is a gradual decrease in numbers until very few active forms are present.

(4) The greatest numbers of protozoa developed sooner in culture solutions inoculated with the largest quantities of soil.

(5) Per gram of soil, there is the greatest development from the least amount of soil used for inoculations.

(6) For the development of all forms soil extract seemed to be a little more favorable than dried-blood extract.

(7) The flagellates are the first organisms to excyst.

(8) Very few large and small ciliates developed as compared with the numbers of flagellates.

(9) Drying the soil slightly favored the development of flagellates in soil extract, while with dried blood there was little difference.

(10) More large and small ciliates developed from the less composted soils.

(11) In dried blood more flagellates developed from the more heavily manured soils.

(12) Very many different types of ciliates were present, while the types and numbers of amœbæ were few.

III.—PROTOZOA OF FIELD AND GREENHOUSE SOILS

INTRODUCTION

The work reported in Part II of this paper on the protozoa of greenhouse soils led the writer to make a more complete investigation of the development of these organisms in culture solutions. In earlier experi-

ments it was found that the development of the different types of protozoa varied greatly with the culture solutions employed in studying these organisms.

The purpose of this problem was to study:

(1) The development of protozoa in different culture solutions.

(2) The development of protozoa from varying amounts of soil inoculations.

(3) The comparison of the numbers and types of protozoa developed from compost and field soils.

A large sample of the same 20 per cent compost greenhouse soil containing 24.30 per cent of moisture which was used in the work on greenhouse soils in Part II was collected from a bin in the greenhouse. Likewise a sample of a heavy clay field soil which had not received fertilizer for several years was collected from a young orchard. This soil, which was taken 3 inches from the surface, had a moisture content of 18.35 per cent, and the temperature at the time of collection was 0.5° C. To 100 c. c. portions of 3 per cent dried-blood extract with 0.05 per cent of dibasic potassium phosphate and the same amount of a 10 per cent hay infusion [1] were inoculated with 1, 5, 20, 50, and 100 gm. portions of each soil. The inoculated solutions were examined for protozoa and then incubated at 22° C. for a period of 30 days. At the same hour each day counts were made of the organisms developed. The examinations were made under the low power of the microscope and the organisms enumerated by the improved loop method as described in Part I of this paper. When the specially prepared slide was not used, in counting culture solutions containing more than 350,000 organisms per cubic centimeter the loop of culture solution was transferred to a plain glass slide, a 5 mm. square of which was carefully ruled off into 40 to 50 small fields of equal size. All the organisms in the incomplete fields on the outer portions of the film of culture solution were counted, the average of the numbers in several fields were taken, and the numbers of organisms calculated as in the improved loop method. This method checked very closely with counts made by means of the special slide.

As in the previous work, the same difficulty of distinguishing large bacteria from small flagellates was encountered. Hence, to facilitate the enumeration of the organisms it was thought advisable to make all of the counts under the low power of the microscope. In many cases it was difficult to distinguish small ciliates from flagellates; hence, notwithstanding precautions taken in enumerating the organisms, it is quite probable that some small ciliates might have been counted as flagellates, and vice versa.

[1] This formula was recommended by N. Kopeloff, H. Clay Lint, and David A. Coleman in 1915, in an unpublished manuscript entitled, "A New Method for the Counting of Protozoa and Some Media for Their Development."

In this study the same classification of protozoa was made as in previous experiments. The small ciliates included all organisms from the smaller to and including *Colpidium colpoda*. The vorticellæ were also included. The large ciliates included all forms larger than *Colpidium colpoda*. The flagellates included all forms of flagellates that were observed.

DEVELOPMENT OF PROTOZOA IN CULTURE SOLUTIONS INOCULATED WITH VARIOUS AMOUNTS OF SOIL

DEVELOPMENT OF SMALL CILIATES

From Table IX it is apparent that the development of small ciliates varies greatly with the kind of media and soil used. It is noted that but few of these organisms developed in any of the solutions of dried-blood extract inoculated with the varying quantities of field soil. Very many appeared from the 5-gm. and 20-gm. inoculations of the same soil in hay infusion, indicating that only definite types develop under certain conditions. That this is true is again apparent. In hay infusion inoculated with 100 gm. of field soil only a few organisms developed on the second and third days. In the 1-gm. and 50-gm. inoculations no small ciliates had developed during the whole period of 30 days, but in the case where 100 c. c. of hay infusion had been inoculated with 5 gm. of soil, as many as 30,700,000 organisms per gram were present on the eighth day. With the 20-gm. inoculations 3,700,000 had appeared on the ninth day. In both media more small ciliates developed from the compost than from the field soil. In the compost inoculations of both dried blood and hay infusion a few organisms appeared on the second day where the largest quantities of soil had been used. But the maximum numbers were not reached until the fourth day with the former and the fifth day with the latter solution. In the development of the maximum numbers of small ciliates in the different culture solutions, a variation in the period of the development was very apparent. In all inoculations of dried blood the greatest numbers were noted on the third and fourth days. With hay infusion the maximum numbers did not appear until the fifth, and in some cases not until the eighth day, depending upon the amount of soil used for inoculation.

DEVELOPMENT OF LARGE CILIATES

No large ciliates were noted in any of the inoculations of the field soil, indicating that either the conditions in the media must have been unfavorable for their development or that there were no cysts of large ciliates in the soil.

That such cysts are present in greenhouse soil is indicated by the development of these organisms in both dried blood and hay infusion. The former solution, however, did not seem to be very favorable for their

TABLE IX.—*Comparison of the numbers of large and small ciliates and flagellates which developed daily in different artificial culture solutions inoculated with varying quantities of field and greenhouse soils for a period of 30 days. These numbers have all been calculated on the basis of 1 gm. of dry soil*

Solution No.	Quantity of soil.	Kind of soil.	Medium.	Kind of organisms.	Days after inoculation.									
					1	2	3	4	5	6	7	8	9	10
	Gm.			Small ciliates					63,000	125,500	125,500	188,500	63,000	63,000
801	1	Field	Blood extract	Large ciliates										
				Flagellates		151,500	10,052,000	9,700,000	8,020,000	125,500	125,500	627,000	1,070,000	525,000
				Small ciliates										
802	5	do	do	Large ciliates										
				Flagellates	14,300	515,000	5,200,000	14,000,000	13,200,000	6,400,000	3,020,000	835,000	1,245,000	1,009,000
				Small ciliates			3,610		3,090					3,210
803	20	do	do	Large ciliates										
				Flagellates		15,150	2,100,000	1,400,000	940,000	707,000	290,000	323,000	72,600	21,000
				Small ciliates						1,235	1,235	2,235	1,285	1,285
804	50	do	do	Large ciliates										
				Flagellates		2,990	202,000	382,000	212,000	78,300	123,000	2,470	2,570	1,285
				Small ciliates										
805	100	do	do	Large ciliates										
				Flagellates		5,250	151,500	172,000	62,300	351,000	234,000	113,000	71,700	2,285
				Small ciliates				245,000	68,000		204,000			
821	1	Greenhouse	do	Large ciliates								136,000	136,000	136,000
				Flagellates		1,215,000	29,200,000	72,200,000	612,000	544,000	272,000	136,000	475,000	403,000
				Small ciliates			65,000	1,515,000	13,400	13,400				
822	5	do	do	Large ciliates										
				Flagellates	15,450	342,000	3,930,000	130,000	26,800	94,700	40,200	26,800	216,000	1,330,000
				Small ciliates			36,800	12,400			3,400			3,400
813	20	do	do	Large ciliates							3,400			3,400
				Flagellates	8,150	24,500	630,000	122,500	54,700	6,800	44,300	10,200	10,200	6,800
				Small ciliates			98,500	131,000			4,120	1,340	1,340	
814	50	do	do	Large ciliates								1,340		1,340
				Flagellates	30,300	321,000	33,000	117,400			5,460	5,460	5,450	1,340
				Small ciliates		2,360	2,360	3,200	2,680	1,340				670
815	100	do	do	Large ciliates										
				Flagellates	42,200	474,000	19,550	6,500			1,900	1,340	4,330	2,340
				Small ciliates										
821	1	Field	Hay infusion	Large ciliates										
				Flagellates		314,000	236,000	16,950,000	33,600,000	62,000,000	707,000,000	27,800,000	39,400,000	40,200,000
				Small ciliates						117,500	1,825,000	30,700,000	6,950,000	1,970,000
822	5	do	do	Large ciliates										
				Flagellates			236,000	6,700,000	8,950,000	7,300,000	50,800,000	10,700,000	11,900,000	209,800

				Small ciliates		3,960			6,540	65,400	60,000	3,260,000	3,730,000	400,000
823	20	....do....	....do....	Large ciliates										
				Flagellates		19,250	240,000	3,360,000	6,220,000	12,300,000	5,400,000	23,850		
				Small ciliates										
824	50	....do....	....do....	Large ciliates										
				Flagellates		9,420	81,500	500,000	23,600	287,000	882,000	2,310,000	500,000	344,000
				Small ciliates		750	750							
825	100	....do....	....do....	Large ciliates										
				Flagellates			3,100	230,500	3,960	20,900	24,600	1,210,000	1,122,000	78,200
				Small ciliates				141,000	17,100,000	177,500,000	102,720,000	25,550,000	3,700,000	24,800,000
831	1	Greenhouse	....do....	Large ciliates										
				Flagellates	84,500	32,900,000	507,000	73,100,000	211,300,000		57,350,000	13,570,000	3,110,000	5,380,000
				Small ciliates					197,000	305,000		197,000	305,000	940,000
832	5	....do....	....do....	Large ciliates						27,800	15,000,000	9,590,000	75,800,000	70,500,000
				Flagellates	16,050	1,165,000	11,380,000	5,150,000	1,350,000	1,122,000	1,080,000	605,000	765,000	417,000
				Small ciliates			140,000	545,000	665,000	63,200		13,900		7,050
833	20	....do....	....do....	Large ciliates			7,500	7,050	42,800			21,400	7,070	13,900
				Flagellates	4,280	21,400	2,500,000	1,430,000	1,070,000	535,000	385,000	410,000	146,600	101,600
				Small ciliates		1,715	139,000	471,000	400,000	42,700	11,780	11,780		
834	50	....do....	....do....	Large ciliates				7,050	27,800			5,670	5,670	22,450
				Flagellates	1,715	205,300	278,000	750,000	141,000	39,600	26,750	26,750	33,100	13,900
				Small ciliates		2,400	43,800	88,200	94,700	4,170				1,390
835	100	....do....	....do....	Large ciliates					5,670		8,450	4,170	2,330	25,500
				Flagellates	750	21,000	175,500	4,930	7,130					

TABLE IX.—*Comparison of the numbers of large and small ciliates and flagellates which developed daily in different artificial culture solutions inoculated with varying quantities of field and greenhouse soils for a period of 30 days. These numbers have all been calculated on the basis of 1 gm. of dry soil*—Continued

Solution No.	Quantity of soil.	Kind of soil.	Medium.	Kind of organisms.	Days after inoculation.									
					11	12	13	14	15	16	17	18	19	20
	Gm.			Small ciliates										
801	1	Field	Blood extract	Large ciliates										
				Flagellates	371,000	125,500	251,000	125,500	567,000	315,000	126,000	380,000	63,000	251,000
				Small ciliates					22,350	12,350		12,350	12,350	24,700
802	5	do	do	Large ciliates										
				Flagellates	480,000	63,000	75,700	12,350	24,700	63,000	226,500	9,630	226,500	9,270
				Small ciliates										
803	20	do	do	Large ciliates										
				Flagellates	3,210	3,210	6,420	6,420	6,420	9,630	9,630	9,630	3,210	15,150
				Small ciliates										
804	50	do	do	Large ciliates										
				Flagellates	1,285	1,285	3,855	1,285	16,350		2,570	3,855	1,285	2,580
				Small ciliates	647									
805	100	do	do	Large ciliates										
				Flagellates	1,285	3,855	647	647		647	2,285	647	647	
				Small ciliates										
811	1	Greenhouse	do	Large ciliates	68,000		68,000							
				Flagellates	68,000	68,000		136,000	136,000	1,174,000	136,000	340,000	136,000	136,000
				Small ciliates									13,400	13,380
812	5	do	do	Large ciliates										
				Flagellates	243,000	325,000	340,000	26,800	216,000	243,000	203,000	81,500	135,000	135,000
				Small ciliates			3,900				3,400			
813	20	do	do	Large ciliates										
				Flagellates	3,400		3,400	3,400	6,800	170,000	6,800		6,800	6,800
				Small ciliates	1,340	1,340			2,680		1,340		1,340	
814	50	do	do	Large ciliates										
				Flagellates	24,700	1,340	4,120	4,120	2,680	6,800	2,680	1,340	5,350	2,680
				Small ciliates		1,340	620							620
815	100	do	do	Large ciliates										
				Flagellates	620	1,340	1,340	1,340	620	1,340	620	1,963	1,340	1,340
				Small ciliates										
821	1	Field	Hay infusion	Large ciliates										
				Flagellates	76,700,000	36,400,000	26,550,000	6,150,000	1,112,000	197,000	123,000	1,505,000	123,500	246,000
				Small ciliates	131,700	157,500	183,000	104,860	104,860	65,300	131,800	78,200	25,700	38,500
822	5	do	do	Large ciliates										
				Flagellates	657,000	1,860,000	657,000	656,000	78,200					

823	20	do	do	Small ciliates	428,000	46,000	38,500		32,100	3,210	3,210	9,630	3,210	12,850
				Large ciliates	38,500	25,700	6,420	12,850	3,210		3,210	3,210	6,420	3,210
				Flagellates	38,500	25,700								
824	50	do	do	Small ciliates										
				Large ciliates	668,000	530,000	130,500	144,500	49,700	331,000	184,000	226,800	151,000	21,360
				Flagellates										
825	100	do	do	Small ciliates										
				Large ciliates	82,500	60,000	25,700	6,420	6,420	3,210	1,925		642	642
				Flagellates	10,325,000	8,770,000	10,700,000	4,800,000	3,600,000	2,550,000	9,630,000	3,000,000	2,420,000	1,287,000
				Small ciliates										
831	1	Greenhouse	do	Large ciliates	847,000	564,000	282,500	706,000	850,000	70,600	202,000	70,500	70,500	214,000
				Flagellates										
				Small ciliates	170,500			112,500	27,800	903,000	2,420,000	1,632,000	957,000	848,000
832	5	do	do	Large ciliates	1,273,000								321,900	
				Flagellates	130,500,000	37,000,000	8,550,000	338,000	283,000	338,000	604,000	1,000,000	857,000	160,000
				Small ciliates	1,273,000	210,500	49,200	63,000	64,250	49,700	84,000	38,500	31,900	17,139
833	20	do	do	Large ciliates	13,900	7,050			7,060		3,530			
				Flagellates	85,500	45,000	39,600	48,200	16,050	5,670	2,780	4,170	1,390	4,170
				Small ciliates										
834	50	do	do	Large ciliates	950,800	535,800	2,835					640	1,390	642
				Flagellates	19,800	13,900	24,600	13,900	10,590	2,140	3,530		2,780	641
				Small ciliates				1,390		1,390	640	2,030	640	2,033
835	100	do	do	Large ciliates	2,890	4,186								
				Flagellates										

TABLE IX.—*Comparison of the numbers of large and small ciliates and flagellates which developed daily in different artificial culture solutions inoculated with varying quantities of field and greenhouse soils for a period of 30 days. These numbers have all been calculated on the basis of 1 gm. of dry soil*—Continued

Solution No.	Quantity of soil.	Kind of soil.	Medium.	Kind of organisms.	Days after inoculation.									
					21	22	23	24	25	26	27	28	29	30
	Gm.			Small ciliates										
801	1	Field	Blood extract	Large ciliates										
				Flagellates	125,500	188,500	230,700	188,500	125,500	6,450	125,500	251,000	251,000	502,000
				Small ciliates		12,350								
802	5	do	do	Large ciliates										
				Flagellates	37,100	24,700	63,000	63,000	37,100	12,900	12,900	12,900	25,200	24,700
				Small ciliates								3,090		
803	20	do	do	Large ciliates										
				Flagellates	3,090	6,180	9,270	6,180	3,090	6,180	3,090		9,270	6,180
				Small ciliates										
804	50	do	do	Large ciliates										
				Flagellates	3,710	1,290	6,180	3,710	6,180	1,290	3,710	1,290	5,100	1,290
				Small ciliates		647								
805	100	do	do	Large ciliates										
				Flagellates	647	647		1,290	647	647	647		1,290	
				Small ciliates		68,000								
811	1	Greenhouse	do	Large ciliates						68,000				
				Flagellates	68,000		136,000	136,000	136,000				136,000	
				Small ciliates			81,500			13,400	13,400			
812	5	do	do	Large ciliates										
				Flagellates	149,500	68,000	26,800	80,400	80,400	108,000	108,000	121,500	26,800	26,800
				Small ciliates		3,400	3,400							3,400
813	20	do	do	Large ciliates										
				Flagellates	3,400	6,800	3,400	10,200	13,500	6,800	3,400	6,800		
				Small ciliates				1,340	1,340					
814	50	do	do	Large ciliates										
				Flagellates	1,340	5,390	2,680	6,800	6,800	5,390	1,340	2,680	1,340	2,680
				Small ciliates										
815	100	do	do	Large ciliates										
				Flagellates		620	620	620		1,349			620	620
				Small ciliates										
821	1	Field	Hay infusion	Large ciliates										
				Flagellates	492,000	123,000	370,000	1,275,000	2,630,000	2,750,000	1,830,000	2,287,000	25,650,000	63,200,000
				Small ciliates	38,500	51,400	25,700	38,500	12,850	12,850	25,700	12,850	12,850	12,850
822	5	do	do	Large ciliates										
				Flagellates					25,700	25,700	12,850	38,500		12,850

823	20	do	do	Small ciliates	12,850	12,850	105,000	36,400	6,420	3,210	6,420		3,270	3,210
				Large ciliates										
				Flagellates						6,420		10,950	9,630	25,700
824	50	do	do	Small ciliates										
				Large ciliates										
				Flagellates	2,560	3,850	3,850	1,286	7,700	2,560	2,560	1,285	1,285	
825	200	do	do	Small ciliates										
				Large ciliates										
				Flagellates		642	6,530	1,920	1,920	642	1,285	1,285	642	642
827	1	Greenhouse	do	Small ciliates	990,000	566,000	1,765,000	424,000	565,000	282,000	282,000	141,000	282,000	141,000
				Large ciliates	212,000	212,000	212,000	212,000	282,000	70,700	141,000	70,700	70,700	70,700
				Flagellates	353,000	212,000	42,800	212,000	282,000	141,000				141,000
				Small ciliates	1,000,000	381,000	42,800	13,900	14,000					
832	5	do	do	Large ciliates					13,900	13,900	13,900	70,600	13,900	112,300
				Flagellates	84,300	84,300	42,800	310,000	707,000	7,060	7,060	10,590	3,530	3,530
				Small ciliates	169,500	42,300	14,000	7,060	7,060					
833	20	do	do	Large ciliates										7,060
				Flagellates	7,060	7,060	3,530	7,060	7,060	10,590	3,530	3,530	7,060	
				Small ciliates	1,390	1,390	4,170	1,390						
834	50	do	do	Large ciliates										
				Flagellates	4,170	1,390	4,170		2,780	4,170	38,300	315,000	4,170	1,390
				Small ciliates	642	642			642	1,390	9,033	3,530		
				Large ciliates		1,390	4,170							
835	100	do	do	Flagellates	2,780	2,780	1,390	4,920	17,000	29,700	41,700	25,480	33,800	28,250

development, as only a few appeared with some of the inoculations on the seventh to the eleventh day after inoculation. In hay infusion, however, they began to appear on the third day; the maximum numbers were reached on the fifth to the sixth day after inoculation; then the numbers decreased.

DEVELOPMENT OF FLAGELLATES

That there were more flagellate cysts in the soils examined or that the media employed were more favorable for the development of these organisms than for ciliates is clearly seen by the fact that in all the culture solutions these organisms were more numerous. There was a great variation in the development of these organisms in the different solutions when inoculated with different soils. Upon noting the development of these organisms from field soil it is seen that in the dried-blood extract they appeared the first and second days after inoculation, the maximum number being reached on the third and fourth days. In hay infusion they appeared the second and third days; the maximum numbers were present from the sixth to the eighth day.

In inoculations of both media with greenhouse soil the flagellates appeared on the first day, the greatest numbers appearing from the second to the fourth day in the blood extract and from the third to the eleventh days in the cases of the inoculated solution of hay infusion. In comparing the period of maximum development of flagellates from the various compost soil inoculations, it is seen that with large amounts of soil inoculations the greatest development was attained several days before the maximum had been reached with the smaller inoculations. In total numbers of organisms developing there was no greater majority from either soil examined. In some solutions of dried-blood extract and hay infusion the greatest numbers were developed from field soils, while the greatest development in others was from greenhouse soil.

DEVELOPMENT OF ALL FORMS OF PROTOZOA

For the development of all forms of protozoa hay infusion was more favorable than dried-blood extract. In the former solutions the bacteria seemed to thrive and multiply much more readily than in the latter. Thus, if protozoa feed upon these organisms, favorable conditions for the development of bacteria might have stimulated protozoan life. In comparing the numbers of all forms of protozoa present it is seen that the maximum development is always present at an earlier period in the solutions with the largest inoculations. This is in all probability due to the presence of many more cysts in solutions to which larger amounts of soil were added.

In considering the development of protozoa on the gram-inoculation basis more than one hundred times as many organisms excysted from the smaller than the larger inoculations of soil. This fact may be due

to the increased toxicity produced from the decomposition products of bacteria and other organisms. It is very clearly noted that in all inoculated solutions as soon as the maximum development is reached there is a gradual decrease in numbers of all types of organisms. In so far as observations have been made, this fact is attributed to several causes: The soil contains various kinds and numbers of living organisms as well as spores and many cysts. If the temperature and other conditions, such as sufficient desirable food, the absence of harmful toxins, and the presence of favorable reactions (acidity or alkalinity) are present when a culture solution is inoculated with the soil, certain types of protozoa as well as other organisms excyst and multiply until conditions become unfavorable. Owing to the lack of desirable food for certain species of organisms, the presence of certain decomposition products of either bacteria, yeasts, molds, or protozoa, or the direct destruction by other forms, the organisms either encyst or die. The organisms probably remain inactive until conditions again become favorable, when they excyst and multiply as before.

TYPES OF PROTOZOA DEVELOPED IN DIFFERENT CULTURE SOLUTIONS

In hay-infusion solutions inoculated with the greenhouse soil the organisms observed corresponded to *Vorticella* spp., *Colpoda cucullus*, *Colpidium colpoda*, *Prorodon ovum* Ehrb., and *Glaucoma* sp. The first two mentioned appeared the second day after inoculation; the others were first noted on the sixth day. In some solutions a few of these forms were still present on the thirtieth day. No vorticellæ developed from any inoculations with field soil, indicating that this form inhabits rich moist soil. This fact has already been noted by several investigators. In hay infusion several different unidentified types developed. This has never before been observed in any of the other culture solutions. A large type of flagellates developed on the third day after inoculation. In some solutions of hay infusion these forms appeared soon after the smaller ones had disappeared. Colpoda was the most common form of ciliate in inoculations with field soil. From inoculations of field soil types of ciliates developed different from those in solutions to which greenhouse soil was added. It was also noted that fewer types of organisms were developed from the former than from the latter soil.

In hay infusion the large ciliates were markedly different from those which had already been observed with dried-blood extract. In the former solution inoculated with greenhouse soil but one type was observed. It was very apparent that dried-blood extract was the most favorable for the development of many types of large ciliates.

On the ninth day after the inoculation in hay infusion to which field soil had been added millions of very minute organisms that appeared to be flagellates were noted. These could barely be recognized under the low power of the microscope. This form was not counted, but was

observed to be present throughout the experiment. Similar protozoa appeared on the fourteenth day in hay infusion to which greenhouse soil had been added.

In hay infusion it was very apparent that as the numbers of protozoa decreased the number of cysts increased. That all the protozoa do not encyst when conditions become unfavorable is shown by the fact that very many dead forms of Glaucoma and Prorodon were seen.

On the twentieth day species of Euglena appeared in dried blood inoculated with field soil and in hay infusion to which greenhouse soil had been added. The latter solution was favorable for the development of these organisms, as 600 per gram of soil appeared on the twentieth day, while on the thirtieth day 28,000 organisms of *Euglena* spp. per gram were counted.

That the soils examined contained a small number of amœbæ cysts or that the conditions in the culture solutions were unfavorable for their development was judged by the fact that very few were observed. From the soil inoculations in dried blood no forms of amœbæ were recognized, while in the case of the hay infusion to which greenhouse soil had been added a few were observed on the twenty-first day.

SUMMARY OF PART III

Under the conditions of this experiment and of Part II it is apparent that in developing protozoa from the soil in artificial culture solutions different numbers and types of protozoa will be developed for every variation in the amounts of each soil used for inoculation and with every culture solution used.

IV.—EFFECT OF TEMPERATURE UPON THE DEVELOPMENT OF SOIL PROTOZOA

INTRODUCTION

In the earlier experiments recorded in Parts II and III of this article it was shown that the development of the numbers and types of soil protozoa in artificial culture solutions varied with the kind of culture solutions as well as with the quantity, physical condition, and kind of soil used for inoculation.

The problem under discussion deals with the development of the numbers and types of soil protozoa which appear at various temperatures in artificial culture solutions inoculated with soil of different origin.

That different conditions of temperature affect the development of protozoa in the soil was recorded by Cunningham (3, p. 14), who, after inoculating a quantity of soil for a period of nine days at 5 to 7° C., then increasing the incubation temperature to 22° C., noted an increase in the numbers of protozoa developed after seven days. "Exposure to a temperature of 30° C. for seven days has caused a fall in the total numbers but a distinct rise in the number of cysts."

A sample of the 20 per cent compost greenhouse soil which had been used in previous experiments was collected. Likewise another sample of the heavy unfertilized clay soil used in another study was brought to the laboratory. A third sample, a light loamy soil which contained 12.19 per cent of moisture and which had received an application of 20 tons of barnyard manure per acre for the last 20 years, was collected 3 inches below the surface. The upper 1¾ inches were frozen, but the temperature at 3 inches was 1.5° C. at the time of sampling.

Four 200 c. c. Jena Erlenmeyer flasks containing 100 c. c. portions each of 3 per cent dried-blood extract with 0.05 per cent of dibasic potassium phosphate and the same number of flasks containing 100 c. c. portions of a 10 per cent hay infusion were inoculated with 5-gm. samples of the newly collected moist soils. (It was found in Parts II and III that on the gram basis a greater development of protozoa can be produced with smaller amounts of soil.) The inoculated solutions were examined for living protozoa, and then one flask of each solution of hay infusion and blood extract inoculated with each soil was incubated at temperatures of 5 to 7° C., another set at 15 to 16° C., a third at 22 to 23° C., and a fourth at 29 to 30° C., for a period of 30 days. At the same hour each day these solutions were examined and the living protozoa counted by the improved loop method under the low power of the microscope.

In order that the inoculated solutions should not vary in temperature during examination, they were kept in constant-temperature baths. In like manner, to guard against excessive evaporation from the solutions inoculated at 29 to 30° C., each flask was placed in a container of water and covered with a large beaker. To prevent variation through the possible effect of excessive light, the flasks were screened in all cases during the incubation period.

As in the previous experiments, the classification of protozoa that was followed in this problem was as follows: The small ciliates included all organisms from the smallest to and including *Colpidium colpoda*. The vorticella type were also included. The large ciliates included all forms larger than *Colpidium colpoda*. The flagellates included all the forms of flagellates that were observed.

DEVELOPMENT OF SOIL PROTOZOA IN CULTURE SOLUTIONS AT VARIOUS TEMPERATURES

DEVELOPMENT OF SMALL CILIATES

That the varying condition of temperature is a very important factor in the development of soil protozoa in culture solutions is noted by the marked variation in the numbers of organisms present. For the development of small ciliates a temperature of 6 to 7° C. proved very unfavorable, as in none of the inoculated solutions did the organisms exceed 265,000 per gram. At this temperature blood extract seemed to be more

TABLE X.—*Number of small ciliates, large ciliates, and flagellates developed daily at various temperatures in different culture solutions inoculated with the same amount of different soils for a period of 30 days. These numbers have all been calculated on the basis of 1 gm. of dry soil*

Solution. No.	Kind of soil.	Medium.	Temperature of incubation.	Kind of organisms.	Days after inoculation.									
					1	2	3	4	5	6	7	8	9	10
1111	Greenhouse...	Blood extract.	6 to 7° C...	Small ciliates...										
				Large ciliates...										
				Flagellates...	15,500	15,500	15,500	77,500	930,000	373,000	652,000	3,500,000	4,460,000	15,200,000
1112	.....do.....	Hay infusion.	.....do.....	Small ciliates...										
				Large ciliates...										
				Flagellates...										
1113	Field.....	Blood extract.	.....do.....	Small ciliates...										
				Large ciliates...										
				Flagellates...					53,100	451,000	571,000	1,205,000	1,735,000	4,380,000
1114	.....do.....	Hay infusion.	.....do.....	Small ciliates...										
				Large ciliates...										
				Flagellates...							279,000	584,000	318,000	438,000
1115	Field+manure	Blood extract.	.....do.....	Small ciliates...										
				Large ciliates...										
				Flagellates...		14,020		70,200	942,000	8,350,000	19,700,000	4,920,000	4,930,000	92,400,000
1116	.....do.....	Hay infusion.	.....do.....	Small ciliates...										
				Large ciliates...										
				Flagellates...					42,200	1,670,000	4,000,000	2,998,000	3,230,000	11,450,000
1121	Greenhouse...	Blood extract.	15 to 16° C...	Small ciliates...					27,450	96,000	278,000			
				Large ciliates...						13,700	13,700	13,700		
				Flagellates...	13,680	13,680	136,800	7,150,000	3,770,000	2,425,000	412,000	27,400	178,000	123,200
1122	.....do.....	Hay infusion.	.....do.....	Small ciliates...					93,200	497,000	435,000	404,000	497,000	435,000
				Large ciliates...										
				Flagellates...		15,500	2,220,000	2,250,000	11,330,000	12,350,000	14,200,000	32,600,000	102,000,000	73,900
1123	Field.....	Blood extract.	.....do.....	Small ciliates...					13,000					
				Large ciliates...										
				Flagellates...			208,300	900,000	677,000	221,000	1,497,000	3,050,000	8,480,000	8,900,000
1124	.....do.....	Hay infusion.	.....do.....	Small ciliates...										
				Large ciliates...										
				Flagellates...		14,820		1,097,000	3,560,000	2,580,000	218,700	178,000	32,600,000	6,650,000
				Small ciliates...					12,340	24,680	61,700	37,020	37,100	37,100
1125	Field+manure	Blood extract.	.....do.....	Large ciliates...						12,340	24,680	86,380	173,000	420,000
				Flagellates...		61,800	1,122,000	7,900,000	8,590,000	7,770,000	1,777,000	642,000	147,000	161,000
				Small ciliates...						27,300	14,050	337,000	281,000	83,400
1126	.....do.....	Hay infusion.	.....do.....	Large ciliates...										
				Flagellates...			407,000	5,820,000	101,000,000	45,700,000	47,400,000	213,500,000	94,400,800	35,200,000
1131	Greenhouse...	Blood extract.	22 to 23° C...	Small ciliates...			88,500	202,000	63,200	25,300		12,650		
				Large ciliates...				70,800		12,600		12,650		
				Flagellates...	25,300	50,600	872,000					25,300	12,620	

1132	....do....	Hay infusion....	do....	Small ciliates....	....	....	53,200	321,000,000	26,900,000	82,000,000	9,900,000	24,200,000	6,440,000	7,226,000
				Large ciliates....	....	....	....	....	....	....	233,000	....	....	13,266
				Flagellates....	....	80,000	239,000	7,400,000	15,970,000	35,500,000	6,440,000	206,000	366,000	1,448,000
1133	Field....	Blood extract....	do....	Small ciliates....	....	....	....	....	....	....	....	....	....	....
				Large ciliates....	....	12,200	988,000	9,150,000	5,600,000	12,080,000	1,645,000	2,170,000	2,168,000	440,000
				Flagellates....	....	....	....	....	50,700	126,000	928,300	915,000	3,900,000	1,288,000
1134	....do....	Hay infusion....	do....	Small ciliates....	....	....	....	....	....	....	....	....	....	....
				Large ciliates....	....	....	6,150,000	27,050,000	9,150,000	790,000	736,000	1,370,000	179,500
				Flagellates....	....	....	92,800	82,000	46,000	11,550	....	11,550	....	....
1135	Field+manure	Blood extract....	do....	Small ciliates....	....	11,550	....	....	....	....	....	....	11,550	....
				Large ciliates....	....	1,560,000	6,060,000	5,460,000	602,000	1,540,000	567,000	2,392,000	276,000	43,200
				Flagellates....	11,550	....	....	600,000	2,020,000	361,000	216,000	433,000	66,000	180,000
1136	....do....	Hay infusion....	do....	Small ciliates....	....	....	128,000	12,000	16,900,000	236,000,000	100,300,000	6,240,000	6,400,000	1,584,000
				Large ciliates....	....	....	....	....	....	....	....	....	432,000	....
				Flagellates....	....	....	....	....	....	....	....	....	....	....
				Small ciliates....	....	12,600	75,800	12,600	12,600	....	....	12,600	12,600	12,610
1141	Greenhouse....	Blood extract....	29 to 30° C.	Large ciliates....	12,600	25,200	....	12,600	12,600	50,400	12,600	37,800	12,610	25,240
				Flagellates....	....	139,800	30,800,000	108,600,000	22,530,000	23,200,000	13,050,000	4,680,000	586,000	452,500
1142	....do....	Hay infusion....	do....	Small ciliates....	26,600	53,200	3,810,000	5,850,000	4,430,000	1,223,000	936,000	5,070,000	172,000	39,900
				Large ciliates....	....	....	....	....	....	....	....	....	....	12,200
				Flagellates....	....	....	....	12,280	....	....	....	....	....	....
1143	Field....	Blood extract....	do....	Small ciliates....	....	61,000	159,200	....	....	....	....	97,600	170,300	330,000
				Large ciliates....	....	....	....	....	....	....	....	....	....	....
				Flagellates....	....	....	....	915,000	1,685,000	750,000	701,000	475,000	3,000,000	1,913,000
1144	....do....	Hay infusion....	do....	Small ciliates....	....	....	....	185,000	543,000	139,000	219,300	185,000	81,000	104,000
				Large ciliates....	....	....	....	....	....	....	....	....	....	....
1145	Field+manure	Blood extract....	do....	Small ciliates....	....	....	....	46,200	211,100	....	11,550	34,650	335,000	243,000
				Flagellates....	....	....	11,550	9,130,000	12,100,000	26,250,000	5,480,000	288,000	106,000	72,000
1146	....do....	Hay infusion....	do....	Small ciliates....	....	....	24,850	....	....	....	....	....	....	....
				Large ciliates....	....	....	....	....	....	....	....	164,000	377,000	36,000
				Flagellates....	....	12,000	4,820,000	33,790,000	13,600,000	5,020,000	1,655,000	....	....	....

TABLE X.—*Number of small ciliates, large ciliates, and flagellates developed daily at various temperatures in different culture solutions inoculated with the same amount of different soils for a period of 30 days. These numbers have all been calculated on the basis of 1 gm. of dry soil*—Continued

Solution No.	Kind of soil.	Medium.	Temperature of incubation.	Kind of organisms.	Days after inoculation.									
					11	12	13	14	15	16	17	18	19	20
1111	Greenhouse	Blood extract.	6 to 7° C.	Small ciliates	.....	31,000	.....	93,000	15,500	72,600	155,200	77,600	46,600	15,500
				Large ciliates	.....	.....	.....	15,500	.....	15,500	15,500	15,500	.....	.....
				Flagellates	32,280,000	19,800,000	15,320,000	20,600,000	11,240,000	2,140,000	3,630,000	947,000	248,000	792,000
1112	do	Hay infusion	do	Small ciliates	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
				Large ciliates	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
				Flagellates	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
1113	Field	Blood extract.	do	Small ciliates	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
				Large ciliates	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
				Flagellates	7,950,000	10,740,000	17,550,000	15,550,000	47,100,000	52,900,000	48,750,000	17,400,000	20,100,000	35,400,000
1114	do	Hay infusion	do	Small ciliates	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
				Large ciliates	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
				Flagellates	982,000	372,000	119,500	398,500	93,000,000	39,500	584,000	6,550,000	11,460,000	13,220,000
1115	Field+manure	Blood extract.	do	Small ciliates	.....	.....	.....	13,650	13,650	.....	13,650	13,050	.....	26,000
				Large ciliates	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
				Flagellates	12,540,000	520,000	266,300	323,000	687,500	590,000	1,193,000	1,430,000	872,000	2,635,000
1116	do	Hay infusion	do	Small ciliates	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
				Large ciliates	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
				Flagellates	3,050,000	844,000	14,950,000	42,300,000	24,200,000	11,820,000	50,600,000	26,600,000	66,000,000	61,300,000
1121	Greenhouse	Blood extract.	15 to 16° C.	Small ciliates	.....	.....	.....	.....	.....	.....	27,400	13,700	13,700	27,300
				Large ciliates	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
				Flagellates	6,850,000	27,400	356,000	1,180,000	850,000	493,000	41,100	13,680	.....	.....
1122	do	Hay infusion	do	Small ciliates	404,000	1,580,000	2,200,000	902,000	232,500	590,000	295,000	186,000	105,500	201,500
				Large ciliates	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
				Flagellates	65,300,000	70,200,000	95,500,000	32,200,000	51,700,000	57,900,000	27,800,000	15,960,000	14,250	761,000
1123	Field	Blood extract.	do	Small ciliates	26,600	26,600	104,000	79,800	39,100	39,100	13,000	52,000	13,000	26,000
				Large ciliates	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
				Flagellates	18,550,000	19,750,000	27,400,000	14,370,000	6,300,000	8,320,000	7,760,000	352,000	456,000	195,100
1124	do	Hay infusion	do	Small ciliates	118,600	148,000	118,600	714,000	1,066,000	13,900,000	14,100,000	43,300,000	45,400,000	8,700,000
				Large ciliates	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
				Flagellates	187,500,000	21,700,000	12,800,000	104,000,000	40,600,000	10,120,000	27,600,000	38,800,000	30,800,000	8,350,000
1125	Field+manure	Blood extract.	do	Small ciliates	24,720	.....	24,720	.....	12,360	.....	.....	.....	24,720	12,320
				Large ciliates	123,260	24,720	24,720	12,360	24,720	.....	.....	12,360	.....	12,360
				Flagellates	468,000	370,000	49,400	49,400	12,330	24,720	24,720	24,720	61,800	61,800
1126	do	Hay infusion	do	Small ciliates	19,100,000	9,620,000	6,210,000	3,090,000	1,090,000	422,000	309,800	575,000	1,095,000	337,000
				Large ciliates	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
				Flagellates	293,000,000	210,000,000	5,780,000	20,380,000	1,333,000	4,850,000	14,000	182,500	506,000	126,300
1131	Greenhouse	Blood extract.	22 to 23° C.	Small ciliates	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
				Large ciliates	.....	.....	.....	.....	.....	.....	.....	.....	12,620	.....
				Flagellates	.....	.....	12,620	12,620	.....	.....	.....	12,620	.....	.....

1132	....do....	Hay infusion	....do....	Small ciliates	6,150,000	4,950,000	4,840,000	4,340,000	2,300,000	3,600,000	3,940,000	2,940,000	1,143,000	412,500
				Large ciliates	531,000		13,266		13,266					26,000
				Flagellates	635,000	39,930	26,540	26,540		13,266	13,266	13,266	13,266	
				Small ciliates			13,180	13,180						
1133	Field	Blood extract	....do....	Large ciliates	513,000	256,000	122,000	146,200	195,000	134,600	134,600	121,000	24,000	48,860
				Flagellates	1,880,000	1,614,000	2,700,000	860,000	539,000	241,000	420,000	89,000	101,500	139,960
				Small ciliates										
1134	....do....	Hay infusion	....do....	Large ciliates	76,200	12,700	12,700	38,100			25,400			
				Flagellates		13,550					13,550			
				Small ciliates										
1135	Field+manure	Blood extract	....do....	Large ciliates	82,000	625,000	596,500	170,000	10,350,000	1,320,000	5,370,000	600,000	680,000	55,000
				Flagellates	192,400	84,000	36,000	12,000	12,000	12,600	24,000		24,000	24,160
				Small ciliates										
1136	....do....	Hay infusion	....do....	Large ciliates	6,340,000	1,271,000	810,000	815,000	747,000	2,283,000	288,000	168,000	264,400	60,000
				Flagellates										
				Small ciliates	12,620	12,620	25,240	12,620	12,620					
1141	Greenhouse	Blood extract	29 to 30° C.	Large ciliates	50,480	37,860	86,000	46,000	37,860	251,440	106,300	53,150	25,240	139,000
				Flagellates	4,930,000	53,150			26,000	53,150			13,300	79,860
				Small ciliates										
1142	....do....	Hay infusion	....do....	Large ciliates		39,900	24,400	12,200	13,300	13,300	13,300	13,300	26,600	26,600
				Flagellates	12,200	12,200			36,600	24,400	13,300	12,200	12,200	12,200
				Small ciliates										
1143	Field	Blood extract	....do....	Large ciliates	207,000	109,000		12,200	24,400	36,600	12,200		12,200	12,200
				Flagellates				85,900						
				Small ciliates			50,800		240,600	12,700	25,400		12,700	12,700
1144	....do....	Hay infusion	....do....	Large ciliates	1,657,000	533,000	58,000	46,400	23,150	23,150	23,150	23,150		
				Flagellates	96,500	58,000								
				Small ciliates					69,000	46,300	46,300	34,750	104,000	11,580
1145	Field+manure	Blood extract	....do....	Large ciliates	57,800	194,200	23,150	11,570						12,000
				Flagellates	66,000	12,000	24,000	12,000	12,000					
				Small ciliates										
1146	....do....	Hay infusion	....do....	Large ciliates	24,000				12,000					
				Flagellates										

TABLE X.—*Number of small ciliates, large ciliates, and flagellates developed daily at various temperatures in different culture solutions inoculated with the same amount of different soils for a period of 30 days. These numbers have all been calculated on the basis of 1 gm. of dry soil*—Continued

Solution No.	Kind of soil.	Medium.	Temperature of incubation.	Kind of organisms.	Days after inoculation.									
					21	22	23	24	25	26	27	28	29	30
1111	Greenhouse...	Blood extract.	6 to 7° C...	Small ciliates...	31,000	15,500	15,500	.....	15,500	.....				
				Large ciliates...	15,500	.....	.....	.....	31,000	.....				
				Flagellates.....	1,440,000	155,000	948,000	575,000	1,147,000	1,426,000				
1112	.....do.....	Hay infusion.	.....do.....	Small ciliates...	.....	.....	.....	.....	.....	.....	46,600	.....	.....	263,200
				Large ciliates...	.....	.....	.....	.....	.....	.....	77,800	93,200	622,000	.....
				Flagellates.....	.....	.....	.....	.....	.....	.....	119,200	39,800	.....	26,500
1113	Field.....	Blood extract.	.....do.....	Small ciliates...	.....	.....	.....	.....	.....	.....	199,000	79,600	225,000	66,400
				Large ciliates...	1,000,000	2,080,000	1,800,000	504,000	278,200	412,000	.....	.....	.....	.....
				Flagellates.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
1114	.....do.....	Hay infusion.	.....do.....	Small ciliates...	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
				Large ciliates...	.....	.....	.....	.....	.....	.....	82,400,000	134,000,000	74,800,000	106,500,000
				Flagellates.....	21,670,000	53,400,000	66,500,000	21,940,000	108,800,000	25,080,000	70,300	28,000	70,300	14,000
1115	Field+manure	Blood extract.	.....do.....	Small ciliates...	.....	42,200	14,000	.....	56,000	28,000	.....	.....	.....	.....
				Large ciliates...	.....	.....	.....	.....	.....	.....	42,200	2,160,000	618,000	518,000
				Flagellates.....	1,402,000	450,000	1,837,000	772,500	1,010,000	870,000	.....	.....	.....	.....
1116	.....do.....	Hay infusion.	.....do.....	Small ciliates...	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
				Large ciliates...	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
				Flagellates.....	97,000,000	50,800,000	53,600,000	33,600,000	35,300,000	61,800,000	53,200,000	53,650,000	73,000,000	95,500,000
1121	Greenhouse...	Blood extract.	15 to 16° C...	Small ciliates...	13,650	13,650	27,300	13,650	27,300	13,650	27,300	13,650	27,300	27,300
				Large ciliates...	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
				Flagellates.....	.....	13,650	54,600	13,650	13,650	13,650	54,600	40,950	54,600	.....
				Small ciliates...	1,128,000	885,000	232,500	1,740,000	201,500	388,000	450,000	278,500	404,000	263,400
1122	.....do.....	Hay infusion.	.....do.....	Large ciliates...	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
				Flagellates.....	130,500	482,000	171,000	46,700	.....	15,500	62,200	46,500	46,500	.....
				Small ciliates...	26,000	26,000	39,000	26,000	39,000	13,000	26,000	39,000	26,000	.....
1123	Field.....	Blood extract.	.....do.....	Large ciliates...	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
				Flagellates.....	169,500	52,000	13,000	13,000	91,000	52,000	39,000	78,000	78,000	65,000
				Small ciliates...	862,000	4,880,000	9,110,000	4,570,000	1,216,000	1,352,000	460,000	1,365,000	1,274,000	1,335,000
1124	.....do.....	Hay infusion.	.....do.....	Large ciliates...	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
				Flagellates.....	550,000	1,230,000	505,000	89,200	59,400	14,800	14,800	29,600	356,500	312,000
				Small ciliates...	24,640	160,500	.....	49,400	8,880,000	8,280,000	5,480,000	5,160,000	5,550,000	135,500
1125	Field+manure	Blood extract.	.....do.....	Large ciliates...	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
				Flagellates.....	210,000	24,720	729,000	99,000	.....	.....	24,720	37,000	172,500	24,720
				Small ciliates...	210,400	547,000	420,800	280,000	280,000	98,500	238,800	56,150	295,000	309,000
1126	.....do.....	Hay infusion.	.....do.....	Large ciliates...	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
				Flagellates.....	252,500	604,000	5,880,000	2,272,000	13,050,000	9,870,000	6,770,000	6,950,000	1,305,000	478,000
1131	Greenhouse...	Blood extract.	22 to 23° C...	Small ciliates...	.....	.....	.....	.....	12,620	.....	.....	.....	.....	12,620
				Large ciliates...	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
				Flagellates.....	12,620	25,240	12,620	12,620	12,620	37,860	25,240	12,620	25,240	12,620

1132	....do....	Hay infusion	....do....	Small ciliates	728,000	680,000	728,000	379,000	172,500	49,000	26,600	26,600				
				Large ciliates	13,300	13,300			13,300	13,300	13,300			13,300		26,600
				Flagellates	13,300	26,600			13,300	13,300				39,900		
1133	Field	Blood extract	....do....	Small ciliates												
				Large ciliates	61,000	36,600	122,200	127,000	13,300	24,400	24,400	24,400		24,400		24,400
				Flagellates	76,200	101,300	101,300		165,000	88,800	88,800	127,000		152,000		139,590
				Small ciliates												
1134	....do....	Hay infusion	....do....	Large ciliates			12,720		12,700		25,400			13,720		50,800
				Flagellates			12,560		23,120	34,730	11,360	23,120				
				Small ciliates												
1135	Field+manure	Blood extract	....do....	Large ciliates	254,400	23,120	11,360	110,000	23,120	237,200	127,000	24,700		115,600		89,400
				Flagellates	13,950		11,360	12,050								13,950
				Small ciliates												
1136	....do....	Hay infusion	....do....	Large ciliates	36,970	72,140	132,000	96,200	252,300	613,600	1,058,000	337,000		2,039,000		204,800
				Flagellates												
				Small ciliates		12,600					12,600					12,600
				Large ciliates												
1147	Greenhouse	Blood extract	29 to 30° C.	Flagellates		75,600	12,600		37,800	25,200		25,200		25,200		13,300
				Small ciliates	12,600	40,000	13,300	26,600	40,000	26,600	79,800					13,300
				Large ciliates	146,100	13,300	13,300				13,300	13,300		13,300		13,300
1142	....do....	Hay infusion	....do....	Flagellates	13,300	13,300	13,300	13,190	39,900	53,200		39,900		26,600		
				Small ciliates	26,600	11,360	12,200	24,400	12,200	12,200	12,200			280,000		53,200
				Large ciliates												
1143	Field	Blood extract	....do....	Flagellates	12,200	12,200	24,400				12,200	24,400		24,400		
				Small ciliates												
				Flagellates												
1144	....do....	Hay infusion	....do....	Small ciliates		12,700					12,700	12,700				
				Flagellates						34,610						
				Small ciliates												
1145	Field+manure	Blood extract	....do....	Flagellates	23,000	34,500	11,500		57,500	23,000	11,500	11,500		23,000		23,000
				Small ciliates	12,000	96,000	12,000	72,000	36,000	24,000	24,000	24,000				
				Large ciliates												
1146	....do....	Hay infusion	....do....	Flagellates						12,000						

favorable than hay infusion. That the conditions in the solutions examined were unfavorable for the existence of these organisms is seen by the fact that they did not appear until the twelfth to the fourteenth day, while in others they did not develop throughout the entire period of 30 days.

With the exception of solutions of hay infusion inoculated with greenhouse and fertilized field soil, in all inoculations of both media greater numbers of small ciliates developed at 15° to 16° C. than at any other temperature, indicating that with these culture solutions this temperature is the most favorable for the development of small ciliates. With every soil examined there was a much greater development of these organisms in hay infusion than in blood extract. These facts indicate that only certain types of small ciliates exist and develop at a definite temperature and that the extent of their development is influenced by the amount of desirable food and other favorable conditions in the culture solutions. Because of the fact that the development of small ciliates in dried-blood extract at 15° to 16°, 22° to 23°, and 29° to 30° C. was not as great as in the solutions of hay infusion, the maximum numbers were in most cases developed sooner in the former than in the latter solutions. At 15° to 16° C. in inoculated solutions of both media the period of maximum development varied considerably. In the inoculation of dried-blood extract with greenhouse soil the greatest numbers were reached on the seventh day, while with the other soils a longer time was required. The maximum development with the field-soil inoculations was on the thirteenth day. With the fertilized field soil the greatest number was not reached until the twenty-fifth day after inoculation. In solutions of hay infusion with greenhouse soil the maximum number of organisms was reached on the thirteenth day, with the fertilized field soil on the eleventh day, and with the field soil on the nineteenth day after inoculation.

Incubating at higher temperatures as a rule encouraged a more rapid development of these organisms. However, this fact was not universal, but it was noted that at the temperatures of 22° to 23° and 29° to 30° C. the small ciliates began to appear at an earlier period than at lower temperatures of inoculation. This fact is especially marked in the greenhouse and fertilized-soil inoculations of both media. At 29° to 30° C. in the greenhouse-soil inoculations the organisms appeared on the second day, at 22° to 23° C. on the third day, at 15° to 16° C. on the fifteenth, and at 6° to 7° C. in one inoculation on the twelfth day, and with the other they did not appear until the twenty-seventh day. A very similar circumstance was noted with the fertilized field soil, but in this case at the highest temperature the organisms did not appear until the third day, in solutions inoculated at 15° to 16° C. on the fifth to sixth day, while in solutions inoculated at 6° to 7° C. they appeared on the four-

teenth day, and in the other solution they did not appear at all. For the development of the greatest numbers of small ciliates at the different temperatures, in the media inoculated with the various soils, the greatest numbers developed in hay infusion at 29° to 30° C. with inoculations of greenhouse and manured field soil, while with field soil the maximum development was at 15° to 16° C.

DEVELOPMENT OF LARGE CILIATES

On examining Tables X, XI, and XII it at once becomes apparent that either the soils examined contained very few large ciliate cysts or the conditions in the media were unfavorable for their existence, as very few of these organisms developed. Blood extract seemed a little more favorable than hay infusion. In all the inoculations with the greenhouse soil a few large ciliates developed. In solutions of dried-blood extract with the greenhouse soil at 6° to 7° they first appeared on the fourteenth day, at 15° to 16° on the sixth, at 22° to 23° on the fourth, and at 29° to 30° C. they did not appear until the ninth day after inoculation. At 22° to 23° and 29° to 30° C. a few large ciliates developed in the hay-infusion inoculations of greenhouse soil. With the exception of a few organisms that appeared in the blood-extract inoculations of the manured field soil at 15° to 16° and in the hay-infusion inoculations of field soil at 29° to 30° C. and in the one mentioned above, no large ciliates appeared in any of the solutions.

DEVELOPMENT OF FLAGELLATES

Again the temperature plays a very important rôle in the development of flagellates. Upon considering the largest numbers of organisms developed at different temperatures in dried-blood extract, it is seen that in all cases the greatest development appeared at 6° to 7° and the smallest at 29° to 30° C. There were 150 to 250 times as many developed at the former temperature as at the latter. In comparing all inoculations of hay infusion it is noted that the greatest development for all the soils examined was at 15° to 16°; the smallest in some cases occurred at 6° to 7°; in others at 29° to 30°. With one exception, at 6° to 7°, greater numbers were developed in hay infusion than in dried-blood extract. Comparing the greatest numbers of flagellates developed at the different temperatures and in the various media, it is seen that the greatest development for all soils was in hay infusion at 15° to 16° C. In all cases in the inoculations of hay infusion and in most cases with dried-blood extract at 6° to 7° these organisms did not appear until the fifth day and the maximum was not reached until the twenty-eighth or twenty-ninth day after inoculation.

TABLE XI.—*Period of the maximum development and number of small ciliates, large ciliates, and flagellates at different temperatures in dried-blood extract and hay infusion inoculated with the same amount of the various soils*

SMALL CILIATES

Soil.	Incubated at 6° to 7° C.		Incubated at 15° to 16° C.		Incubated at 22° to 23° C.		Incubated at 29° to 30° C.		Incubated at 6° to 7° C.		Incubated at 15° to 16° C.		Incubated at 22° to 23° C.		Incubated at 29° to 30° C.	
	Dried-blood extract.	Days after inoculation.	Dried-blood extract.	Days after inoculation.	Dried-blood extract.	Days after inoculation.	Dried-blood extract.	Days after inoculation.	Hay infusion.	Days after inoculation.	Hay infusion.	Days after inoculation.	Hay infusion.	Days after inoculation.	Hay infusion.	Days after inoculation.
Greenhouse	155,200	17	178,000	7	88,500	3	75,800	3	263,200	30	2,200,000	13	82,000,000	6	108,600,000	4
Field	119,200	27	104,000	13	12,180	14	36,600	15	.....	.....	45,400,000	19	3,960,000	9	88,900	14
Field + manure	70,300	27	8,880,000	25	92,800	4	543,000	5	.....	.....	19,100,000	11	2,020,000	5	26,250,000	6

LARGE CILIATES

Greenhouse	31,000	25	13,700	6	70,800	4	12,620	9	.....	.....	.....	.....	53,200	11	26,600	29
Field	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	12,700	27
Field + manure	.....	.....	420,000	10	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....

FLAGELLATES

Greenhouse	32,180,000	11	7,150,000	4	872,000	3	139,000	20	622,000	29	102,000,000	9	25,500,000	6	5,850,000	4
Field	52,900,000	16	27,400,000	13	12,880,000	6	330,000	10	134,600,000	28	187,500,000	11	27,900,000	5	3,000,000	9
Field + manure	92,400,000	10	8,540,000	5	10,350,000	15	335,000	9	97,000,000	21	293,000,000	11	236,000,000	5	33,700,000	4

DEVELOPMENT OF SOIL PROTOZOA AT DIFFERENT TEMPERATURES

From the data presented it is seen that the maximum number of small ciliates in the dried-blood extract were found at 15° to 16° C., while the greatest number of flagellates appeared at 6° to 7° C. In hay infusion the flagellates developed in greater numbers at 15° to 16°. In some solutions of hay infusion the small ciliates developed at 15° to 16° and in others at 29° to 30° C.

That the ciliates were directly detrimental to the development of flagellates can not be definitely stated at this time, but it is noted that in solutions of culture media incubated at 6° to 7° C., where very few ciliates developed when the flagellates had excysted, many more were present until the end of the experiment than had been noticed in any other inoculations. In view of this fact the flagellate development might be influenced by the presence of ciliates. At 6° to 7° C. the maximum number of flagellates appeared from 7 to 17 days before the maximum ciliate development was reached. At this temperature in no case was the maximum ciliate development noted until the tenth or eleventh day, while the greatest ciliate development appeared from the seventeenth to the twenty-seventh day after inoculation. In all inoculations of hay infusion at 6° to 7° the maximum number of flagellates developed at an earlier period than did the ciliates. The maximum development of the former occurred from the twenty-first to the twenty-ninth day, while that of the latter was 30 days after inoculation. In all inoculations at 15° to 16° the maximum development of small ciliates and flagellates was reached sooner than at the temperature of 6° to 7° C. This period varied from 2 to 20 days. In comparing the maximum numbers of small ciliates and flagellates developed at 22° to 23° with those developed at 15° to 16° it is seen that, with two exceptions, the greatest development was reached earlier at 22° to 23° C. Comparing the development at 22° to 23° with that at 29° to 30°, it is seen that there was no uniformity; in some cases the maximum was reached sooner at the former, while in others it occurred at the latter temperature. As in previous experiments, when the maximum development was reached, the numbers of protozoa gradually decreased, showing that the conditions for continuous multiplication became unfavorable.

Table XII shows the variation in the rate of multiplication of the protozoa in the different solutions from their first appearance until the first increase was noted. This increase was usually the first day after their presence was noted. There is a marked difference in the rate of multiplication of each type in each solution at every different temperature employed. No correlation is noted between the multiplication of the small ciliates in like infusions inoculated with the same soil incubated at different temperatures. The same statement holds true for the multiplication of the flagellates and large ciliates. In like

manner there is no correlation between the multiplication of the different protozoa (all types) in the different infusions inoculated with the same soil and incubated at the same temperature. For nearly all inoculations the greatest multiplication of the flagellates is noted at 22° to 23° C. In some solutions the small ciliates multiplied faster at 15° to 16°, in others at 22° to 23°, and in still others at 29° to 30° C.

TABLE XII.—*Rate of multiplication of the protozoa in culture solutions from the time of their first appearance until the first increase was noted. This period was usually 24 hours*

INCUBATED AT 6 TO 7° C.

Solution No.	Large ciliates.	Small ciliates.	Flagellates.
1111	2.0	3.0	5.0
1112	.....	6.0	1.2
1113	.....	0	9.0
1114	.....	.....	2.0
1115	.....	2.0	13.0
1116	.....	.....	42.0

INCUBATED AT 15 TO 16° C.

1121	0	4.0	10.0
1122	.....	5.0	148.0
1123	.....	2.0	4.5
1124	.....	1.5	3.0
1125	2.0	2.0	18.0
1126	.....	24.0	14.0

INCUBATED AT 22 TO 23° C.

1131	0	2.3	2.0
1132	4.0	588.0	3.0
1133	.....	0	82.0
1134	.....	2.5	4.5
1135	.....	8.0	136.0
1136	.....	3.3	1,408.0

INCUBATED AT 29 TO 30° C.

1141	0	6.0	2.0
1142	2.0	259.0	2.0
1143	.....	2.0	1.5
1144	0	0	2.0
1145	.....	3.0	4.0
1146	.....	386.0	401.0

If in the future study of soil protozoa quantitative determinations are to be made, definite standards of quantity and of certain character (moist or dry) of soil must be taken in order that all the results will be comparable. Likewise, a definite standard of inoculation temperature and the kind of culture media must be fixed. The time at which the examinations are made must be uniform.

In so far as the data presented in Parts II and III show on the gram basis the greatest development of certain types of small ciliates, large ciliates, and flagellates is secured from 1-gm. inoculations in 100 c. c. of solution. However, 5 gm. of soil would be more representative than 1 gm; and, as shown by Cauda and Sangiorgi (2), on the gram basis

larger numbers of organisms would develop from this quantity than from larger amounts of soil.

In comparing the development of protozoa from moist and dry soils (Part II) very little difference in the development of small ciliates, large ciliates, and flagellates was found, but in order that the conditions of the soil protozoa would not be varied in this experiment moist soil was used.

Cunningham (3, p. 14) found 22° C. the optimum temperature for the development of most soil protozoa. In this problem a great variation was found. The greatest uniformity of results in the development of small ciliates was obtained at 15° to 16°. With flagellates 6° to 7° was the optimum in some solutions and 15° to 16° in others. As compared with the development at 22° to 23°, a much longer period of time was required for the maximum numbers of protozoa to develop at the temperatures lower than 22° to 23°. It may be added that 22° is more convenient to maintain in the laboratory.

In culture solutions the conditions are much different compared to those found in the soil, as was already suggested by Martin (12). Of the solutions examined the hay infusion proved the most satisfactory for the development of large numbers of organisms, the total being much greater than those appearing in the natural soil. It is probable that the results produced by adding the soil to tap water more nearly represents the conditions as they are found in the soil.

In order that the results may be comparable, the examinations of the inoculated culture must be made periodically every day. The length of time a culture should be incubated will vary with the temperature of incubation, kind and amount of soil, and kind of medium. Under certain conditions some types of protozoa appear on the first day after inoculation; these would multiply very rapidly and probably depress the development of other forms. Again, certain types might not appear until the eighteenth or twenty-second day after inoculation. With few exceptions, however, at temperatures of 15°, 22°, and 29° C., the maximum development of small ciliates and flagellates in the culture solutions inoculated with the soils examined was not reached until the thirteenth or fourteenth day. The writer is of the opinion that the development in artificial culture solutions in the first five to seven days would more nearly show the numbers of cysts of small ciliates and flagellates present in the soil.

The conditions under which the experiments have been carried out seemed to have been very unfavorable for the development of large ciliates and amœbæ. In the soils examined, however, cysts of large ciliates could be readily seen under the microscope. Cauda and Sangiorgi (2, p. 396) developed many amœbæ in Giltay's, Omelianski's, Hiltner's, peptone, and mannite solutions, showing that these organisms were present in the soils which they examined.

That the temperature influences the development of the different types of soil protozoa in culture solutions is shown by the fact that the numbers of the different species developed varied a great deal. The forms studied tallied best with the descriptions of *Colpoda cucullus*, *Colpidium colpoda*, *Vorticella* spp., *Prorodon ovum*, and *Glaucoma* sp. They appeared in some of each series of solutions incubated at 15°, 22°, and 29°. Of these organisms *Colpoda cucullus* was the only one that developed at 6°. At this temperature only a few appeared on the fourteenth day, and these were present until the twentieth day. Other ciliates that developed at 6° were *Paramecia* spp. At 15° *Colpoda cucullus* was the most numerous form of the ciliate type. These first appeared on the sixth day and were present throughout the experiment. Incubating at 15° was not very favorable for the development of the vorticellæ. They appeared on the fifth day and were still seen on the thirtieth day after incubation. Besides species of Colpoda, Vorticella, Prorodon, and Glaucoma, long slender ciliates, possibly *Condylostoma patens* Müll., were very numerous at 22° and 29°. At 22° species of Vorticella, Colpoda, Prorodon, and Glaucoma appeared on the third day. The vorticellæ were present until the twenty-sixth day after inoculation, while the other forms were still seen on the thirtieth day. The temperature of 29° was more favorable for the development of *Colpoda cucullus*, *Prorodon ovum*, and *Glaucoma* sp., but at this temperature many vorticellæ were also developed. This last-named form appeared on the second day and was noted until the fifteenth day after inoculation. The most prominent flagellates were species of Monas and Bodos.

As previously noted in Part III, all the small ciliates do not encyst when the conditions become unfavorable. This was again noted, for in the solutions incubated at 29° many dead individuals of *Prorodon ovum* and *Glaucoma* sp. were seen on the seventh day after inoculation.

The higher temperatures of incubation were the most favorable for the early excystment of small ciliates.

That the Vorticella cysts are present in field soils which have received applications of manure is shown by the fact that these organisms developed in culture solutions inoculated with the fertilized soil. The numbers, however, were very small as compared to those developed in solutions inoculated with greenhouse soil.

Upon several occasions during the examination of the solutions an individual of *Colpoda cucullus* was seen in the process of being excysted.

The conditions under which this experiment was carried out seemed to be unfavorable for the development of amœbæ, as none of these organisms developed in any of the media employed.

SUMMARY OF PART IV

Under the experimental conditions described above it has been found that:

(1) A temperature of 15° to 16° C. is the most favorable for the development of small ciliates.

(2) At 15° to 16° hay infusion is the most favorable culture solution for the development of small ciliates.

(3) The maximum numbers of small ciliates are developed at an earlier period in dried blood than in hay infusion.

(4) The maximum development of small ciliates at 6° to 7° varies from 17 to 30 days after inoculation.

(5) At 15° to 16° the maximum development of small ciliates occurs from seventh to twenty-fifth day after inoculation.

(6) The small ciliates develop sooner at the higher than at the lower temperatures.

(7) The lower temperatures retard the development of small ciliates.

(8) Dried-blood extract and hay infusion are unfavorable media for the development of large ciliates.

(9) Large ciliates will develop at all the temperatures noted if conditions are favorable.

(10) Many flagellates developed at all temperatures noted.

(11) The maximum development of flagellates occurs at 6° to 7° in dried-blood extract and at 15° to 16° in hay infusion.

(12) Hay infusion is the most favorable medium for the development of maximum numbers of flagellates.

(13) The higher temperatures encourage early development of flagellates, while lowest temperatures retard their development.

(14) At all temperatures the flagellates develop sooner than the ciliates.

(15) At 15° to 16° the flagellates appear four or five days before the ciliates.

(16) As soon as the maximum development is reached there is a gradual decrease in the numbers of all forms of protozoa.

(17) Species of Colpoda, Vorticella, Prorodon, and Glaucoma develop at 15° to 16°, at 22° to 23°, and at 29° to 30°.

(18) A few individuals of Colpoda and Paramecium develop at 6° to 7°.

(19) At 15° to 16° C. Colpoda is the most numerous ciliated form.

(20) The temperature of 29° to 30° is very favorable for the development of species of Colpoda, Prorodon, and Glaucoma.

(21) Some ciliates die when conditions become unfavorable.

(22) Vorticella cysts are present in field soils which have received applications of manure.

(23) Hay infusion and dried-blood extract are unfavorable media for the development of amœbæ.

GENERAL CONCLUSIONS ON THE DEVELOPMENT OF SOIL PROTOZOA IN ARTIFICIAL CULTURE SOLUTIONS

From all data which have been presented in this paper the writer feels that he is warranted in concluding that—

(1) The development of soil protozoa in artificial culture solutions inoculated with the soils which have been examined varies with the conditions of the problem.

(2) The development of soil protozoa in artificial culture solutions varies with the kind of media employed.

(3) For each quantity of soil which is used for inoculation there is a variation in the development of protozoa.

(4) Drying the soil affects the development of soil protozoa.

(5) There is a variation in protozoan development in every greenhouse soil.

(6) Field soil causes differences in the protozoan development.

(7) At each temperature of incubation there is a variation in the development of soil protozoa.

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TYLENCHUS SIMILIS, THE CAUSE OF A ROOT DISEASE OF SUGAR CANE AND BANANA

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OCCURRENCE OF TYLENCHUS SIMILIS IN FIJI AND HAWAII

A serious outbreak of a disease among bananas (*Musa sapientum*) in Fiji in 1890–91 caused the planters great uneasiness. At the request of Sir John Thurston, British High Commissioner of the Pacific, the Department of Agriculture of New South Wales, Australia, undertook an investigation, which was conducted by the writer. Most of the banana plants examined grew in the gardens adjacent to Government House at Suva, Fiji, where experimental plantings were made in connection with the disease. During the investigations roots of the banana and the soil about the roots were examined with a view to discovering possible causes of the disease. It was during this particular part of the investigation that a new species of nematode was discovered, to which the name "*Tylenchus similis*" was applied. Only the male was seen.

Nothing further was discovered concerning this species of Tylenchus until 1907, when, during a visit to sugar plantations on Kauai, one of the Hawaiian Islands, the same nematode was again found by the writer, this time infesting the roots of sugar cane (*Saccharum officinarum*). Both sexes of the nematode were found in abundance, and to these specimens, which at the time appeared to represent a new species, the name "*Tylenchus biformis*" was applied. *T. biformis* proved to be a true parasite and was found to be sufficiently injurious to the roots of sugar cane to justify a careful examination.

The nematode appeared to attack the roots at or near the tips, with the result that the root soon succumbed, thus compelling the plant to throw out new roots, which in turn became infested. The attacks of the nematode resulted in striking lesions, easily discoverable whenever the attacks were of a pronounced character. The tissues of the root lost their white or colorless appearance and took on first a cinnabar-red color, then a reddish purple color. The latter was succeeded by a dark purplish red, and this in turn by a purplish black. The discolored areas were sometimes several millimeters in length. In advanced cases the tissue of the axial part of the root was attacked, and large numbers of the nematodes were readily discovered in the colored cavities caused by their activities. The seriousness of the result was increased by the fact

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that the breach created by the nematodes afforded entrance to fungous and microbic enemies.

It will thus be seen that this Hawaiian species of Tylenchus was found under circumstances conclusively proving its parasitic nature. Every degree of infestation was found in the sugar-cane roots, from those which upon external examination, even with a lens, appeared to be in a sound condition to roots spotted with numerous dark infested areas, each harboring scores of the nematode parasites. Sections of the roots showed that the cavities inhabited by the nematodes were colored or blackened on the inside and that it was this discoloration which gave rise to the outward appearances already described. All stages of the nematode were found in the cavities, including full-grown males and females, and it was plain that this species of Tylenchus lived generation after generation largely in the roots of the sugar cane, though it would undoubtedly be necessary, in the natural course of events, for the progeny sooner or later to remove from one root to another or from one plant to another. It was therefore to be expected that nematodes of this species would be found in soil adjacent to the roots of sugar cane, although the investigations made at the time did not disclose any stage of the parasite living free in the soil.

OCCURRENCE OF TYLENCHUS SIMILIS IN JAMAICA

Recently this nematode disease has been reported from the Island of Jamaica. The following are extracts from letters written by Mr. S. F. Ashby, Microbiologist of the Department of Agriculture, Jamaica:

I send you in a carton some fragments of diseased portions of rhizomes and true stems of the Jamaica (Gros Michel) banana preserved in dilute formalin. The disease, locally called "black head," shows as a black rot working into the tissue from the surface usually from around the insertions of diseased roots; the roots when attacked show depressed dark flocks at the surface, and within the cortex a purple rot disintegrating in the older parts.

The disease is widespread here owing to suckers for planting being frequently dug from affected stools; it is responsible for much backward growth and short bunches on land depending on rainfall in moderate or bad seasons.

Dr. Erwin F. Smith, after an examination of the material accompanying Mr. Ashby's letter, was of the opinion that the disease was not caused by *Fusarium* spp.

DESCRIPTION OF TYLENCHUS SIMILIS[1]

A comparison of the species of Tylenchus found in Hawaii with the other species known at the time seemed to indicate that it was not identical with any form previously described. It was, however, pointed

[1] For an explanation of the formula used in descriptions of nematodes *see* Cobb, N. A., Antarctic Marine Free-Living Nematodes of the Shackelton Expedition, p. 6, Baltimore, 1914 (Contrib. Sci. Nematology, I).

The illustrations were prepared under the author's personal supervision by Mr. W. E. Chambers.

out that the males and females were so unlike that, had they not been found in conjunction and under such circumstances as to preclude the possibility of error in assigning them to one and the same species, it is probable that they would have been considered to be separate species. The remarkable similarity of the male to those of the species of Tylenchus previously found about banana roots in Fiji did not escape notice, but as the Fijian observations were incomplete, no females of the Fijian species having been seen, the question of the identity of *Tylenchus similis* and *Tylenchus biformis* was not raised. The present investigation establishes the identity of these two species. The species should therefore bear the prior name "*Tylenchus similis* Cobb, 1892."

This nematode is, in the opinion of the writer, clearly proved to be the primary cause of a disease of the sugar cane. Mr. S. F. Ashby, in a recent letter, writes as follows concerning its relation to banana:

> I at first attributed the attack [on banana] to the joint action of a Fusarium and a coli-like bacterium frequently isolated from the rot; inoculation of either or both failed to cause a similar rot. Eelworms[1] were always found present, and on going through samples from various sources again I invariably came across the same species in the advance margin of the rot both in rhizomes and roots.

The specimens forwarded by Mr. Ashby contained no other organism that would appear to have caused the lesions.

The following description of *Tylenchus similis* is derived from specimens forwarded from Jamaica by Mr. Ashby in diseased banana tissues.

Tylenchus similis Cobb. *Tylenchus biformis* Cobb, 1907. $\left(\Longrightarrow \frac{3.}{2.5} - \frac{11.5}{3.2} - \frac{18.}{3.4} - \frac{59.-.38}{3.8} - \frac{88.}{2.6} .7 \text{ mm.}\right.$ The moderately thick layers of the transparent, naked, colorless cuticle are traversed by somewhat more than 400 transverse striæ, which are not further resolvable. The transverse striæ are interrupted on the lateral fields by conspicuous wings, the presence of which is indicated by four longitudinal striæ taking up a space equal to one-fourth to one-third the diameter of the body. The two outer of these lines are more conspicuous than the two inner, inasmuch as they are somewhat wider and more refractive. The two inner lines are sometimes faint and occupy about one-fourth to one-fifth the width of the entire wing space. The outer margins of the wings are almost imperceptibly crenate, a feature which is associated with the transverse striæ of the cuticle; the inner lines are also crenate, but even less markedly so. These wings begin opposite the base of the spear, where, however, they are not so pronounced as along the median regions, and extend backward to near the end of the tail. They maintain their maximum development in a rather uniform way from opposite the nerve ring to a little behind the anus. The posterior portion of the neck is subcylindroid, while the anterior portion is convex-conoid, and ends in a rounded head, which in the

[1] Nematodes.

female has a flattish, hemispherical lip region, set off by a more or less distinct constriction. The striæ begin to diminish in size in the neighborhood of the base of the spear, and are only about one-third to one-half as wide at the base of the lips as they are farther back. These transverse striæ are so pronounced a feature that they give to the contour of the body a crenate appearance, especially toward the posterior extremity. The lip region also is minutely transversely striated, the number of labial striæ being about 8 to 10. There are arched radial ceratinous elements in the lip region, but these have not been accurately counted. It seems likely there are about six of them. The mouth opening is very small, and the vestibule is strengthened by ceratinous elements which serve as a guide to the spear. This latter is somewhat longer than the base of the head is wide and in the females at least is a strongly developed and doubtless very efficient organ. It may be divided into two regions the posterior of which is cylindrical, and ends at its hinder extremity in a strongly developed threefold bulb, about one-fourth as wide as the corresponding portion of the head, and to which are attached muscles that pass forward to near the outer portion of the base of the lip region. The anterior half of the spear is narrower, ends anteriorly in a somewhat blunt point, and is hardly half as wide as the larger posterior cylindrical portion. At the base of the spear the œsophageal tube begins. At this point it is about two-fifths to one-half the width of the corresponding portion of the neck. It has this diameter until near the median bulb, where it diminishes in such a way that at the actual junction with the bulb the diameter of the definite constriction separating it from the bulb is only one-fourth to one-sixth that of the neck. The median bulb is fairly well developed in the female, though much deteriorated in the male. In the female it is elongated to ellipsoidal in form, and about two-thirds as wide as the corresponding portion of the neck. It is supplied with a fairly well-developed but somewhat simple refractive valvular apparatus having a diameter nearly one-third as great as that of the bulb itself. Behind the bulb the œsophagus is again narrow—about one-sixth as wide as the corresponding portion of the neck. It soon widens out a little so as to become more than half as wide as the base of the neck. It joins the intestine in a somewhat indefinite manner. The length of the posterior part of the œsophagus may be judged by the fact that the distance from the anterior margin of the median bulb to the end of the œsophagus equals nearly half the length of the neck. In stained specimens the beginning of the intestine is indicated by the special cardiac cells of the intestine, which stain more strongly than the cells immediately behind them (fig. 1). The intestine is made up of cells which are packed with spherical granules of various sizes and of more than one kind. The smallest of the granules of the smaller sort have a diameter considerably less than the width of one of the striæ; the larger are two or three times as wide. The fatty granules or accretions of the intestine, the granules

of the larger sort, are of very much larger size and give to the organ its peculiar pearly appearance.

From the inconspicuous anus the rectum leads inward and forward a distance about equal to the anal body diameter. The tail is conoid to the rather blunt roughly conoid terminus, which has a diameter about one-third ot one-fourth as great as that of the base of the tail. On each lateral line, a little in front of the beginning of the middle third of the tail, there is a minute pore, which is possibly homologous with the single papilla found in the corresponding position on the tail of the male. The final striæ are rather indefinite, so that the terminus appears almost as if not striated. The lateral fields appear to be more than one-third as wide as the body. The excretory pore is rather conspicuous, as is the duct leading to it. Both walls of the duct are distinctly refractive, and its lumen may readily be seen. The pore is located about as far behind the median bulb as the base of the spear is in front of it. The duct leads backward a distance equal to three to four body widths, and there joins the rather small ellipsoidal renette cell located on the left-hand side of the body. The exact details of this renette cell are not yet clear. There is a conspicuous refractive cell of rather uniform granular texture located just behind the excretory pore. This cell is longer than the body is wide, about one-third as wide as long, and has a strongly refractive nucleus about one-fourth as wide as itself. Closely associated with this cell are two others of similar form but somewhat smaller, the three forming a close tandem series twice as long as the body is wide. As a rule, the two posterior cells of this series exhibit peculiarities not shown by the anterior cell; they do not stain so strongly with carmine, and in general are less conspicuous. These three glandular cells empty through a narrow duct which enters the base of the œsophagus in the rear of the nerve ring, passes through the median bulb, being diverted to pass around the central valve on its dorsal side, and extends thence onward to near the

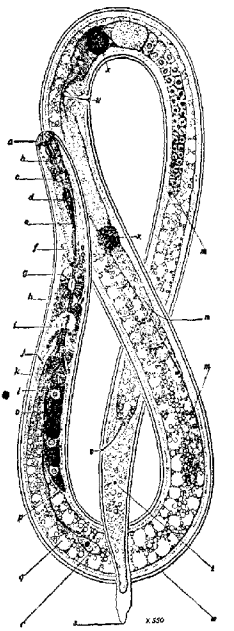

FIG. 1.—*Tylenchus similis:* Nearly adult female. *a*, Lip region; *b*, spear guide; *c*, 3-bulbed base of spear; *d*, ampulla, salivary gland; *e*, œsophageal lumen; *f*, œsophagus; *g*, median bulb; *h*, nerve cells; *i*, nerve ring; *j*, excretory pore; *k*, initial intestinal cells; *l*, anterior salivary gland; *m*, *m*, end of ovary; *n*, ovum; *o*, renette duct; *p*, posterior salivary gland; *q*, fat granule, intestine; *r*, renette cell (?); *s*, terminus; *t*, caudal pore; *u*, vulva; *v*, anus; *w*, crenate cutide; *x*, *x*, spermatozoa.

base of the spear, where the duct enlarges to form a distinct, elongated ampulla, emptying into the œsophageal lumen immediately behind the base of the spear. From the somewhat broadly elevated but otherwise not very conspicuous vulva the vagina leads inward at right angles to the ventral surface fully halfway across the body, where it joins the two uteri, one of which extends forward and the other backward. In the females found infesting sugar-cane roots on the Island of Kauai, in Hawaii, the thin-shelled eggs were observed to be about twice as long as the body is wide and fully five-sixths as wide as the body. They begin segmentation before deposition. The blastomeres are rather coarsely granular.

Male formula. $\left(\longrightarrow 0 \frac{2.4}{1.8} - \frac{12.}{2.6} = \frac{18.}{2.8} - \frac{M\ 12}{2.6} - \frac{89.}{22}\right)$ .7 mm. The male differs in many important respects from the female, not only in the form of the tail end but in that of the anterior extremity as well. The neck of the male tapers rather regularly from the intestine forward, though it decreases rather more rapidly in diameter anteriorly, where it ends in a short, somewhat subcylindrical or hemispherical lip region set off by a deep and distinct constriction. This lip region appears to be composed of about the same number of striæ as that of the female, and to have the same general structure in spite of its difference in form and size. The spear of the male, however, is very weakly developed and is nothing like so efficient an organ as that of the female; in fact, at times it is difficult to convince oneself that the male really possesses an oral spear. From the structure of the mouth of the male it appears somewhat doubtful whether he is able to make his way unaided into the tissues of the host plant. It seems more probable that he works his way into the cavities already created by the voracity of the female. The bulbous base of the spear is no wider than one of the nearby annules of the cuticle, and the shaft at its widest part is considerably narrower than any of the annules of the cuticle. It tapers anteriorly to an excessively fine narrow point. The wings of the male are similar to those of the female, but are hardly so strongly developed (fig. 2). The tail tapers from some distance in front of the anus and diminishes in size rather regularly to near the blunt terminus. The posterior portion is subcylindroid and ends in a bluntly conoid terminus, which is about half as wide as the base of the tail, and which, like that of the female, is not provided with a spinneret. The bursal flaps spring from the submedian lines at a point just in front of the proximal ends of the spicula. When the body is seen in profile, the bursa extends beyond the ventral contour from opposite the proximal ends of the spicula to near the middle of the tail and continues almost to the end of the tail. Near the junction of the middle and anterior thirds of the tail there are two ventrally submedian, finger-shaped papillæ, which extend into the bursa and appear to reach about halfway to its margin. The bursa, like the cuticle, is striated, and its margin is crenate. The

regular striations of the cuticle extend nearly to the terminus. The two equal, slightly arcuate, or nearly straight, tapering spicula are about one and one-third times as long as the anal body diameter. Their proximal ends are cephalated by constriction. At their widest part, which is near the constriction, they are about one-fifth as wide as the corresponding portion of the body. Distally they taper to a slightly blunt point. The accessory pieces are about half as long as the spicula and are placed parallel to them. Passing inward the accessory piece increases in thickness for some distance and then near the middle begins to taper and at the same time to curve away from the spicula almost imperceptibly. The accessory piece appears to have attached to it muscles which pass backward, but the distal attachment of these muscles has not yet been made out. The single testis extends forward and has its tapering blind end located considerably behind the middle of the body. The proximal portion of the testis is about one-half as wide as the corresponding portion of the body. The males are more rare than the females, the ratio appearing to be about 1 male to every 5 to 10 females.

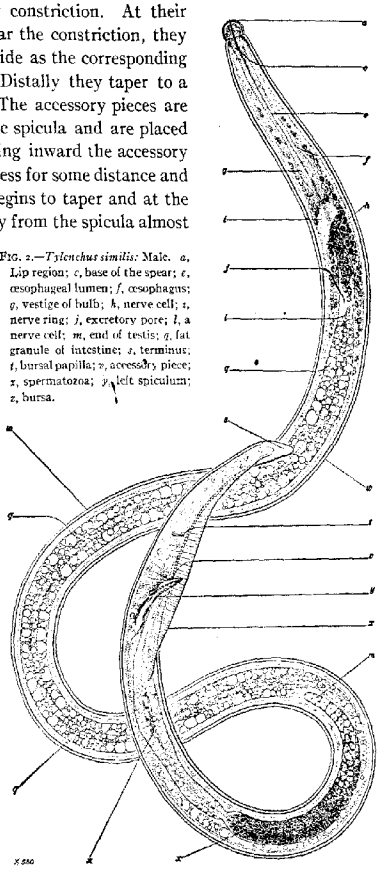

FIG. 2.—*Tylenchus similis:* Male. *a*, Lip region; *c*, base of the spear; *e*, œsophageal lumen; *f*, œsophagus; *g*, vestige of bulb; *h*, nerve cell; *i*, nerve ring; *j*, excretory pore; *l*, a nerve cell; *m*, end of testis; *q*, fat granule of intestine; *s*, terminus; *t*, bursal papilla; *v*, accessory piece; *x*, spermatozoa; *y*, left spiculum; *z*, bursa.

It is a rather remarkable feature of this species that the young have tails more blunt than the adults, the reverse being usually the case with nematodes.

Habitat. (1) About the roots of bananas (Fiji); (2) in the roots of sugar cane (Kauai, Hawaii); (3) in the roots of bananas (Jamaica).

CONCLUSION

On the basis of our present knowledge it is impossible to suggest the original habitat of this nematode. In view of its habits, its known distribution indicates that it is adapted to tropical and subtropical conditions of widely different character. Its infestation of plants differing from each other so widely as banana and sugar cane leads to the suspicion that it may be another addition to the already formidable list of nematode parasites which adapt themselves to a great variety of conditions. Its presence in Jamaica suggests the possibility of its introduction thence into Porto Rico and the southern portions of the mainland of the United States, where it would probably find suitable host plants in the sugar cane and might be expected to attack other plants.

In one way this investigation of the anatomy of *Tylenchus similis* adds materially to our knowledge of the group of Tylenchi to which it belongs. For a long time observers have noted in this group the presence of puzzling tissues or organs near the base of the neck, and these have been described and figured in a way that indicated a very incomplete and unsatisfactory knowledge of their real nature; in fact, they have always been regarded simply as constituents of the cardiac bulb. These researches prove that in *Tylenchus similis* these peculiarities of the base of the neck are due to the presence of a threefold gland emptying through the lumen of the œsophagus near the base of the spear.

What appear to be homologous organs are known in other genera and are regarded as "salivary glands," admittedly more on the basis of their structure than on the results of physiological tests. However, the morphological evidence is very strongly in favor of the conclusions reached.

The presence of such organs has not hitherto been noted in Tylenchus or any nearly related genus. The details of the organ are difficult to follow, but once they had been demonstrated it became evident that a similar organ exists in other species of Tylenchus, and it is especially interesting to note the presence of a similar organ in the well-known *T. dipsaci* Kühn, or, as it is yet more commonly known, *T. devastatrix* Kühn, the devastating nematode, so often responsible in the past for great damage to bulbous crops, such as the onion and hyacinth. This similarity in structure between *T. dipsaci* and *T. similis* makes it all the easier on structural grounds to suspect *T. similis* of becoming a serious pest whenever it gets an opportunity. Whatever may be the cause, there is no doubt of the ability of this species rapidly to break down the tissues of the plants it attacks. One may now suspect, and on very good grounds, that this ability is due not only to the battering action of the oral spear but to the chemical action of a special secretion. Entirely in accord with these ideas is the absence of this organ in the male of *T. similis;* when the oral spear deteriorates, the gland deteriorates also.

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